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Impact of macular pigment optical density on photophobia threshold

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IMPACT OF MACULAR PIGMENT OPTICAL DENSITY ON PHOTOPHOBIA THRESHOLD

BY

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ABSTRACT

IMPACT OF MACULAR PIGMENT OPTICAL DENSITY ON PHOTOPHOBIA THRESHOLD

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Two carotenoids, lutein and zeaxanthin, accumulate in the central retina where they are collectively referred to as macular pigment (MP). By absorbing short-wavelength light, MP may attenuate photophobia (PP)—visual discomfort induced by normal light exposure—for targets composed of short-wavelength light. The aim of this study was twofold: first, to investigate a possible relationship between integrated macular pigment optical density (MPOD) and PP thresholds for short-wavelength targets relative to mid- to long-wavelength targets; and second, to measure changes in PP thresholds after increasing MP with lutein supplements.

MPOD was measured psychophysically at 20′, 30′, 60′ and 120′ eccentricity with a Macular Metrics® densitometer. Each subject's MPOD profile was fit with a Gaussian and Lorentzian function and the area under the curve was calculated to yield an integrated Gaussian MPOD (iGMPOD) or integrated Lorentzian MPOD (iLMPOD). PP thresholds for two foveal and two parafoveal, 8.2-degree targets were measured using a Maxwellian-view optical system. At both loci, a scaling technique was used to measure subjects' level of discomfort for a short-wavelength broadband (blue) light, which was strongly absorbed by MP, and a mid- to long-wavelength broadband (orange) light, which was not absorbed my MP. For both eccentricities, the log relative energy necessary to induce PP for the blue target was subtracted from the log relative energy necessary for the orange

target. The foveal value was then subtracted from the parafoveal value to yield a PP ratio. PP ratios and iGMPOD and iLMPOD were calculated for ten subjects. Repeated measures were obtained for four of these subjects after six weeks and twelve weeks of consuming 60mg of lutein ester supplements per day.

PP ratios were positively correlated with iGMPOD ($r=0.830$, $p=0.002$) and iLMPOD ($r=0.775$, $p=0.008$). According to a subjects-by-trials design, both iGMPOD ($F=9.12$, $p=0.015$) and iLMPOD ($F=17.35$, $p=0.003$) significantly increased from baseline. Significant increases in integrated MPOD corresponded to significant increases in PP ratio ($F=10.41$, $p=0.036$).

The results suggest that MPOD may influence the amount of short-wavelength light necessary to elicit PP. Further, PP thresholds for short-wavelength lights can be increased by augmenting MPOD with lutein supplementation.

CHAPTER I

RETINAL CAROTENOIDS

Carotenoids are a class of naturally derived pigments synthesized by various plants and protists. The basic structure of all carotenoids is a string of isoprene units. The majority of identified carotenoids have a six-carbon ring at each end of a carbon chain, which may include one or more oxygen groups. If the carbon ring contains oxygen, the carotenoid is referred to as a xanthophyll (e.g., lutein and zeaxanthin), otherwise it is called a carotene (e.g., β-carotene).

In plant systems, carotenoids appear to be synthesized to protect the organism via two physiological reactions. First, carotenoids' chain of conjugated double-bonds allows them to transfer energy from singlet oxygen and other free radical species, effectively quenching these noxious species. Second, carotenoids absorb light in the potentially harmful, short-wavelength (SW) region of the spectrum and dissipate the energy as heat. Carotenoids consumed in the diet may play these same beneficial roles in humans and other animals. In fact, epidemiology studies have repeatedly found correlations between carotenoid rich diets and good health. Specifically, carotenoid intake has been linked with a lower risk for atherosclerosis (Dwyer et al., 2001; Gale et al., 2001) as well as various types of cancers, such as prostate cancer (Lindsey 2002; Miller et al., 2002), breast cancer (Johnson, 2000) and ovarian cancer (Huncharek et al., 2001). Dietary carotenoids may also reduce one's risk of developing cataract and age-related macular

degeneration (AMD; Moeller et al., 2000), two leading causes of blindness (Robinson et al., 1997; AREDS, 2000).

Two carotenoids are primarily associated with preventing cataractogenesis and AMD, lutein and zeaxanthin. These two plant pigments are found in a variety of fruits and green, leafy vegetables commonly consumed in the human diet (Khachik et al., 1992a; 1992b; Sommerburg et al., 1998). The absorption of lutein and zeaxanthin occurs in the duodenum, where the carotenoids are released from their food matrices and incorporated into micelles. Some of these micelles may transfer the carotenoids to mucosal cells, via passive diffusion, whereas other carotenoid-containing micelles are unabsorbed (Parker, 1996). After entering the enterocyte, lutein and zeaxanthin are incorporated into chylomicra and then released into the blood stream. The carotenoid concentration in chylomicra peaks approximately four to eight hours after intake (Yeum & Russell, 2002). Circulating chylomicra are degraded by lipoprotein lipase and the chylomicron remnants are collected by the liver. In the liver, carotenoids are repackaged within lipoproteins. Some carotenoids, like β-carotene, are predominately distributed in low-density lipoproteins (LDL), whereas lutein and zeaxanthin are distributed evenly between LDL and high-density lipoproteins (HDL; Yeum & Russell, 2002). The carotenoid concentration in lipoproteins peaks approximately 16 to 48 hours after ingestion (Erdman et al., 1993).

The absorption, or bioavailability, of carotenoids is influenced by several factors (see van het Hof et al., 2000). For example, dietary fat must be consumed concurrently with carotenoids in order to stimulate micelle formation. In the absence of dietary fat, carotenoid absorption may be as low as 5%, whereas a small amount of fat may increase

absorption to approximately 45% (Ribaya-Mercado, 2002). Unlike other carotenoids that may be optimally absorbed when consumed with a limited amount of fat, one study reported that lutein absorption was significantly greater in individuals who consumed a lutein supplement with 34.4g of fat daily for seven-days, compared to individuals who consumed the lutein with 3g of fat (Roodenburg et al., 2000). Another factor that affects the absorption of carotenoids is the food matrix containing the carotenoid. Generally, absorption of carotenoids is greater when they are suspended in an oil solution (i.e., supplements), as opposed to raw vegetables (van het Hof et al., 1999). Processing of raw vegetables, on the other hand, may increase the bioavailability of some carotenoids. Castenmiller et al. (1999) showed that processing raw spinach improved the bioavailability of β-carotene, but had little effect on lutein absorption. The absorption of carotenoids is also affected by the interaction or competition between carotenoids in the lumen and enterocytes. A number of researchers has shown that coincident consumption of lutein with other carotenoids may impair lutein absorption (see van den Berg, 1999). Tyssandier et al. (2002), for example, reported that chylomicron lutein levels were diminished when lutein (from spinach) was consumed with lycopene supplements. Conversely, Gärtner et al. (1996) showed that after consuming a single dose of Betatene™ —a natural extract containing lutein, zeaxanthin, α-carotene and β-carotene— the chylomicron distribution of lutein was fourteen times greater than its relative concentration in the supplement; the zeaxanthin distribution was four times greater than its relative concentration.

Carotenoids are transported through the body in lipoproteins, and via unrealized mechanisms, deposited in lipophilic tissues such as adipose tissue and various immune

organs (Olsen, 1984). Lutein and zeaxanthin, to the exclusion of other carotenoids, cross the blood-retinal barrier and are deposited in the inner plexiform (i.e., ganglion dendrites and bipolar cell extensions) and photoreceptor-axonal layers (Snodderly et al., 1984). Although the exact mechanisms mediating this retinal accumulation are unknown, Bernstein et al. (1997) suggested that specific lutein and zeaxanthin retinal binding proteins, perhaps tubulin, may be responsible.

Lutein and zeaxanthin accumulate throughout the entire human retina (Handelman et al., 1988). Using high-performance liquid chromatography, researchers have estimated that the total retinal concentrations of lutein and zeaxanthin are between 4 and 145ng, with lutein accounting for approximately two-thirds of the combined tissue concentration (Bone et al., 1988; Rapp et al., 2000). These carotenoids, however, are not uniformly distributed in the retina. The aggregate concentration of both carotenoids is greatest in the macular region, hence they are collectively called the macular pigment (MP). According to Bone et al. (1988), who measured retinal lutein and zeaxanthin concentrations between 0mm and 12.2mm from the foveal center, over 50 percent of the retina's macular carotenoids accumulate within one-degree ($\approx$ 0.33mm) of the central point. Lutein and zeaxanthin have two different concentration gradients. In the fovea, the concentration of zeaxanthin exceeds that of lutein at a ratio of about 3:1 (Landrum et al., 1999). In contrast, lutein is the dominant carotenoid in the peripheral retina, accounting for two-thirds of the total concentration. Edge et al. (1997) suggested that the higher concentration of zeaxanthin relative to lutein in the central retina was due to its greater antioxidant capacity. Other researchers have noted the obvious correlation between the distribution of photoreceptors and the differing concentration gradients of

lutein and zeaxanthin (Elsner et al., 1998; Sommerburg et al., 1999). Although both of these hypotheses may be correct, they cannot completely account for the high degree of variance in MP among individuals.

In the last fifteen years, more than thirty investigations have reported the mean macular pigment optical density (MPOD) of various cohorts. The diverse assessment techniques and stimulus parameters make meta-analytical estimates of a population average difficult. However, for twelve studies that employed similar assessment methods, the mean MPOD (at 30 minutes of retinal eccentricity) of approximately 1,200 total subjects ranged between about 0.20 and 0.45. Data tables listed in two of these studies showed that MPOD ranged between 0.01 and 0.5 in one study (Cuilla et al., 2001b), and 0.0 and 0.94 in the other study (Hammond et al., 1997b). Despite the unknown sources of variance, Hammond and his colleagues, in a series of studies, discovered three factors that may contribute to between-subject variability. One, the greater light transmission of blue and gray irises, compared to dark irises, may represent increased photodegradation of retinal lutein and zeaxanthin, in that individuals with light-colored irises tend to have lower MPOD than individuals with dark irises (Hammond et al., 1996b). In their study, the mean MPOD for 38 individuals with blue or gray irises was 0.25, whereas the mean MPOD of 31 subjects with brown or black irises was 0.38 ($p < 0.01$). Similar findings were observed in recent large-scale studies (Hammond & Caruso-Avery, 2000; Cuilla et al., 2001a). Two, cigarette smoking may affect MPOD via depleting available body stores of lutein and zeaxanthin or by increasing pro-oxidant activity in the retina (Hammond et al., 1996c). These researchers found that, on average, smokers had approximately half the MPOD of nonsmokers, 0.16 versus 0.34. Three,

males tend to have higher MPOD than females (Hammond et al., 1996a). In that over 75 percent of the body's supply of carotenoids are found in adipose tissue (Olsen, 1984), it may be that females' greater adiposity provides additional binding sites for carotenoids (Su et al., 1998), resulting in less available lutein and zeaxanthin. That is, adipose tissue may compete with the retina for carotenoids. It follows, then, that both males and females with high amounts of adipose tissue might have lower MPOD. To wit, Hammond et al., (2002) reported that obese individuals in their sample had significantly lower MPOD than individuals with a BMI (body mass index) less than 29. Although these factors identified by Hammond and colleagues may impact MPOD, several studies have found contrary relationships. For example, in a study by Johnson et al., (2000), females had significantly higher MPOD, despite their greater adiposity. In another study by Beatty et al., (2001), there was no relationship between MPOD and either smoking status or iris color. Nonetheless, it is interesting to note that the factors associated with lower MPOD in the Hammond studies are also correlated with greater risk for developing AMD.

CHAPTER II

AGE-RELATED MACULAR DEGENERATION

AMD is the leading cause of irreversible legal blindness among the aged population in the United States (National Advisory Eye Council, 1998). About twenty-percent of the population over the age of 60 have an early form of AMD called age-related maculopathy (ARM; Klein et al., 1992; Cruickshanks et al., 1997), and between five and eight-percent (six to ten-million Americans) have developed macular degeneration (Beatty et al., 1999; la Cour et al., 2002). The hallmark of AMD pathophysiology is the accumulation of extracellular debris between the retinal pigment epithelium (RPE) and Bruch's membrane. The extracellular debris, called drusen, consists of various cellular materials (e.g., unphagocytized disc membranes) thought to be excreted by dysfunctional RPE cells (Hogan, 1972). As extracellular deposits accumulate, RPE cells are stretched, depigmented and may eventually die, ultimately resulting in photoreceptor necrosis (Young, 1987). Drusen formation occurs with age, with most individuals over the age of 50 having drusen in at least one eye (Fine et al., 2000). A few small (< 64μm), yellow drusen with demarcated borders, so called "hard" drusen, are usually asymptomatic and not a strong risk factor for AMD. In the Beaver Dam Eye Study, for example, only one individual with exclusively hard drusen developed AMD (Klein et al., 1997). In contrast, "soft" drusen are large (> 64μm), pale yellow, have poorly defined boundaries, and are a potential risk factor for AMD (Zarbin, 1998). When the number or size of drusen significantly impair central vision, often a

visual acuity of 20/200 or worse, the individual is said to have atrophic (dry) AMD. In 15% of individuals with dry AMD, a more devastating loss of vision occurs when new choroidal blood vessels grow and penetrate the weakened Bruch's membrane (Goldsmith, 2003). These fragile blood vessels often hemorrhage, resulting in extensive photoreceptor death. This latter form of AMD is called "wet" or exudative AMD.

A number of risk factors for ARM and AMD have been identified in recent studies. In addition to genetic susceptibility (Zarbin, 1998) and age (Beatty et la., 2001), some *potential* risk factors for AMD include: light-colored irises (Sandberg et al., 1994; Pratt, 1999), light exposure (Young, 1988; van der Hagen et al., 1993), female sex (Cruickshanks et al., 1993; la Cour et al., 2002), BMI (Schaumberg et al., 2001) and cigarette smoking (Seddon et al., 1996; Evans, 2001). Another important factor that receives a great deal of attention is diet. Several studies have found positive correlations between AMD and fat consumption (Cho et al., 2001), and inverse correlations between macular insult and carotenoid intake (Snodderly, 1995; Mares-Perlman et al., 2001). For example, Snellen et al. (2002) showed that the prevalence of AMD was almost twice as high in individuals who consumed a low-lutein diet, compared to individuals with a lutein rich diet. Given the similarity between the factors correlated with low MPOD and the risk factors associated with AMD, as well as the possible inverse relationship between dietary carotenoid intake and macular damage, numerous researchers have postulated that the MP may protect the retina.

CHAPTER III

PROTECTIVE ROLE OF MACULAR PIGMENT

The macular carotenoids may protect the retina and contiguous tissues via the same mechanisms observed in plant systems. One such way in which retinal lutein and zeaxanthin may afford protection is via their ability to quench free radicals. Free radicals are highly unstable molecules due to an unpaired electron in their outermost orbit. To become stable, free radicals must capture an electron from another molecule, in turn, producing another unstable molecule. This initial process can trigger a chain of such reactions, as unstable molecules steal electrons from adjacent molecules—the outcome often being damage to the involved particles. Several oxidation products of lutein and zeaxanthin were discovered in donor retinae by Khachik et al. (1997), suggesting that the macular carotenoids may interact and possibly quench free radicals in the retina.

Free radicals are generated throughout the body, but the presence of high oxygen content and focused SW light make the posterior ocular tissues particularly prone to the generation of these reactive species. For example, lipofuscin, the metabolic byproducts that accumulate in RPE cells with age, are photogenerators of oxygen radicals (Gaillard et al., 1995; Bonnel et al., 2003), perhaps the most deleterious free radical species (van der Hagen et al., 1993). Oxygenated radicals, such as hydroxyl radicals and hydrogen peroxide species, are often used in in vitro analyses to estimate carotenoids' antioxidant efficacy. In short, researchers can determine a carotenoid's relative antioxidant capacity

by measuring the amount of light energy passing through a solution containing free radicals and a specific carotenoid. As carotenoids bind with free radicals and share electrons, they are degraded, resulting in less available pigment to absorb light. Consequently, more light energy passes through the solution. For example, Woodall et al. (1997b) found that in a solution containing peroxyl radicals, there was about a thirty-percent increase in both zeaxanthin's and lutein's transmittance at λ_{max} after ten minutes of incubation. In other words, almost thirty percent of lutein and zeaxanthin concentrations were degraded in ten minutes. In a solution containing hydrogen peroxide, these same researchers found that after five minutes, a little over thirty percent of the total lutein concentration was degraded, and almost sixty-five percent of the zeaxanthin concentration was depleted. This suggests that in the presence of hydrogen peroxide, zeaxanthin may be a more effective antioxidant than lutein. Compared to other carotenoids like β-carotene and lycopene, lutein and zeaxanthin have much slower degradation rates in the presence of free radicals (Mortensen & Skibsted, 1997a; 1997b; Woodall et al., 1997a), suggesting that they are less effective antioxidants. In turn, the rapid free radical degradation or photo-bleaching of other carotenoids (e.g., lycopene) might be the basis for their absence in the retina (Siems et al., 1999). However, other carotenoids, like astaxanthin are more photo-stable than lutein and zeaxanthin, but less effective as antioxidants. It seems, then, that lutein and zeaxanthin's presence in the retina represents an evolutionary compromise between antioxidant effectiveness and photo-stability.

A second way in which MP may protect the retina is via screening SW light. The absorption spectrum of MP resembles a mesokurtic bell-curve, ranging between 410nm

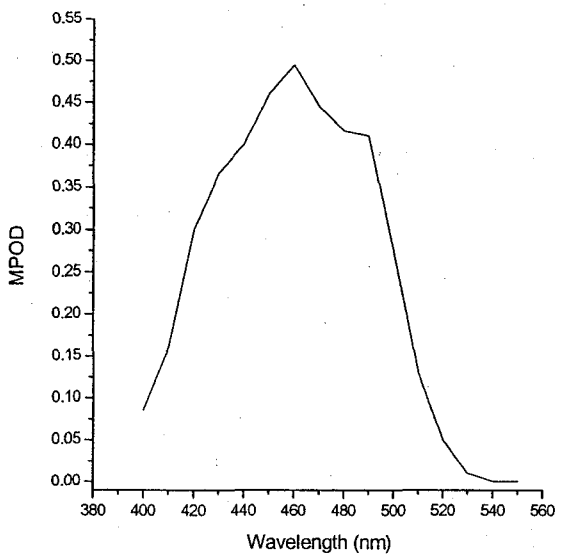

Figure 1: Absorption spectrum of macular pigment measured psychophysically by Wyszecki and Stiles (1982).

and 520nm, with a maximum absorption at 460nm (see Figure 1; Wyszecki and Stiles, 1982). Interestingly, MP absorbs light at the more damaging end of the visible spectrum. Ham et al. (1976) measured retinal damage as a function of intensity and wavelength, and found that approximately hundred times less energy is needed at shorter wavelengths (e.g., 440nm) to produce retinal insult, compared to longer ones (e.g., 590nm). The elevated damage potential of SW light has also been noted by other investigators (Ruffolo et al., 1984). The link between MP's absorption spectrum and the greater ability of SW light to cause damage seems obvious; MP may absorb potentially harmful SW energy and dissipate it as heat, attenuating the amount of light energy reaching the photoreceptors and supportive tissues. Thus, the combined functionality of retinal lutein and zeaxanthin—a SW filter and antioxidant—may protect the macula from light-induced insult (Snodderly, 1995; Pratt, 1999), and thereby sustain visual sensitivity (Hammond et al., 1998).

In addition to protecting the retina against light damage, the filtration effects of MP may help to improve visual acuity. Wooten and Hammond (2002) referred to this possibility as the *acuity hypothesis*. The focusing elements of the human eye (lens and

cornea) inherently produce longitudinal chromatic aberration, such that an emmotropic eye focused on a middle-wavelength (MW) target will bring SW light to a focus anterior to the retina (myopic), and long-wavelength (LW) light to a focus posterior to the retina (hyperopic). This effect is most pronounced for SW light, exceeding –1.0 diopters for wavelengths below 470nm (Wyszecki & Stiles, 1982). The consequences of chromatic aberration on normal viewing conditions are often unnoticed, if not compensated for by the visual system (i.e., down-regulating the response of the short-wave system). Only under experimental conditions are the effects of chromatic aberration apparent. For example, subjects report the presence of a "violet" penumbra when viewing a broad-band target (Hammond et al., 2001). Perhaps the perceptual intensity of this SW fringe is attenuated by the MP. Indeed, Reading and Weale (1974) calculated that the filtration of SW light by MP should lessen the appearance of chromatic distortion. Although there is little empirical evidence to suggest that MP may improve acuity via attenuating chromatic aberration, several studies have reported increased visual function in individuals with macular disease after intervention with spinach (Richer, 1999) and lutein supplements (Dagnelie et al., 2000; Olmedilla et al., 2001).

The SW light filtration of MP may also impact other conditions involving light exposure. For example, Mesri and Dellepiane (1991) investigated the ability of various filters to mitigate the photoconvulsive response in patients with photosensitive epilepsy. They found that SW absorbing filters could lessen or eliminate photoconvulsive responses, whereas filters had little effect. Perhaps the filtering properties of MP could impact patients' thresholds for photoconvulsive responses, such that increased MPOD could improve the quality of life for individuals with photosensitive epilepsy.

CHAPTER IV

PHOTOPHOBIA

MP may also influence the magnitude of discomfort associated with photophobia (PP)—a condition characterized by the exacerbation or generation of pain or discomfort as a consequence of normal light exposure. Although all individuals have likely experienced an acute episode of PP, or dazzling glare (Vos, 2003), as they entered a bright environment after prolonged exposure to dark surroundings (e.g., exiting a movie theater on a sunny day), some individuals suffer from chronic light-induced pain. For example, patients with neurological disorders, such as trigeminal neuralgia (Wolff, 1963), and individuals suffering from eye diseases, like conjunctivitis, retinitis pigmentosa, and AMD (Lebensohn, 1934; 1951; Gawande et al., 1989; Bacotti, 2001), often report persistent hyper-sensitivity to light and periods of PP as symptoms of their condition. PP also appears to accompany migraine headaches (Drummond, 1997; Vanagaite-Vingen & Stovner, 1998; Muelleners et al., 2001), and may be more common among females (Krymchantowski & Moreira, 2001). It should be noted that PP is a clinical term, lacking a standardized operational definition, and is sometimes referred to as photoaversion or discomfort glare in non-clinical literature.

The etiology of visual pain associated with *extreme* light exposure (e.g., snow blindness) is usually the result of UV light induced damage to the corneal epithelium. Under *normal* light exposure, however, the mechanisms mediating visual discomfort or pain are poorly understood. Research in the early 1900s demonstrated that several

functioning anatomical structures were necessary to experience PP. Although the retina is insensible to pain, it seems logical that the photoreceptors play a role in PP. Siegwart (1920) showed that only wavelengths of light within the visible spectrum could produce PP. Further, he showed that individuals with bilateral blindness could not experience visual discomfort in response to light. In three individuals with unilateral blindness, iris constriction and pain was reported in the blind eye when the functioning eye was exposed to light (Siegwart, 1920). Other investigators also demonstrated that iris constriction was involved in PP. For example, in one study, pharmacological dilation of subjects' pupils alleviated the visual discomfort they previously experienced when entering sunlight (Nagel, 1901). These findings suggest the neural pathway responsible for (bilateral) iris constriction may be necessary to experience visual discomfort; in particular, the optic tract projecting to the Edinger-Westphal nuclei in the pretectal midbrain, and the inferior division of the oculomotor nerve (3^{rd} cranial nerve), which connects these nuclei with the iris sphincter. After noting that the iris dilates and constricts with some rate of recurrence when the eye is exposed to bright light (i.e., hippus), Hopkinson (1956) suggested that oscillations of the iris might be involved in visual discomfort. Although research by Howarth and colleagues (1972) failed to corroborate this possibility, an intact trigeminal nerve (5^{th} cranial nerve), connecting to the ciliary body and the iris dilator muscle via the nasociliary and long ciliary nerves, is necessary to experience PP (Lebenson, 1951). Recently, Stringham et al. (manuscript submitted for publication) conducted a series of experiments revealing that small bistratified and parasol ganglion cells may mediate PP. Small bistratified ganglion cells are thought to contribute to the blue-yellow opponent processes, and give rise to the koniocellular pathway (Kremers et al., 2001). Parasol cells

give rise to the magnocellular visual pathway, which is involved in processing depth, movement and is characterized by a luminosity function (Kaplan & Shapley, 1986; Livingstone & Hubel, 1987). Interestingly, some parasol ganglion cells project to pretectal nuclei (Rodieck, 1998), perhaps contributing to iris constriction.

Few studies have investigated the stimulus parameters mediating PP. Instead, most research has focused on a similar phenomenon called discomfort glare—a condition in which distracting, possibly discomforting, light sources are located in the peripheral field of view (Vos, 2003). Most research investigating the stimulus parameters necessary to elicit discomfort glare (or PP) in normal observers has relied on subject self-report. Typically, subjects are asked to rate their visual discomfort in response to a stimulus using the standardized de Boer scale (de Boer, 1967), which ranges from 1 (unbearable) to 9 (just noticeable). Using this scale, Sivak et al. (1999) found that both the intensity and duration of a light source significantly affected observers' ratings of discomfort. As the duration or intensity of the light source increased, so did the apparent level of visual discomfort. Other investigators have reported that discomfort thresholds are lower for binocular targets (Vanagaite et al., 1997; Vanagaite-Vingen & Stovner, 1998), suggesting that visual discomfort may involve binocular summation. Waters et al. (1995) demonstrated that nonuniform stimuli (i.e., gratings) presented in the peripheral retina were less discomforting than nonuniform stimuli viewed centrally.

In 1994, Berman et al. introduced the use of electromyography for assessing discomfort glare. They placed electrodes around the eye and measured muscle contraction in response to light exposure. This "objective" measure of visual discomfort is based on the premise that a discomforting light will trigger a contraction of the

orbicularis muscle, resulting in a reduction of the palpebral fissure (i.e., a squint). For a glare source located at 11 degrees retinal eccentricity, they found that muscle contraction increased as the luminance of the light source increased. Further, their subjects appeared to experience more discomfort when viewing a 2-degree glare source, compared to a 1-degree source. In a subsequent study, Berman and colleagues (1996) used this same objective technique to measure the effects of a light's spectral emmitance on discomfort. They found that a light source with more energy in the LW end of the spectrum induced greater muscle contraction (i.e., discomfort) compared to a light source with more energy at shorter wavebands. A greater discomfort in response to LW light was also noted by Main et al. (2000). According to their research, the discomfort thresholds for both LW and SW light targets are lower than for MW and broad-band targets. Similarly, Kuller and Wetterberg's (1993) subjects reported that full-spectrum fluorescent lights (correlated color temperature > 5000 K) were more discomforting than warm-white fluorescent lights (correlated color temperature = 3000 K). In a recent, more experimentally rigorous investigation, Stringham et al. (2003) looked at the effects of individual wavelengths on the threshold for PP. Unlike other researchers, they controlled retinal illuminace (pupil size) by using Maxwellian view optics. Their procedure involved focusing monochromatic targets, subtending 5 degrees of arc and presented for 5 seconds, on the fovea of three normal observers. The method of limits was used to determine the energy necessary to elicit a criterion squinting response as measured by electromyography at wavelengths between 440nm and 640nm. The normalized action spectra of PP for all three of the observers showed a high degree of between- and within-subject uniformity. Figure 2 shows the normalized action spectra for two subjects. As

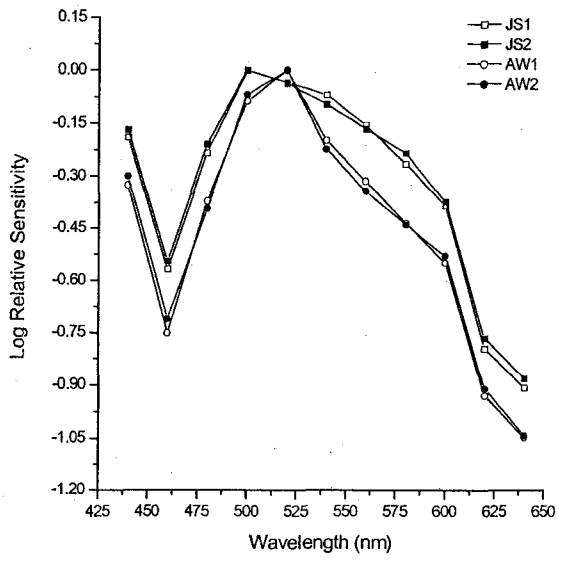

Figure 2: Photophobia action spectra for two subjects from Stringham et al (2003). The two action spectra for each subject are normalized at peak sensitivity.

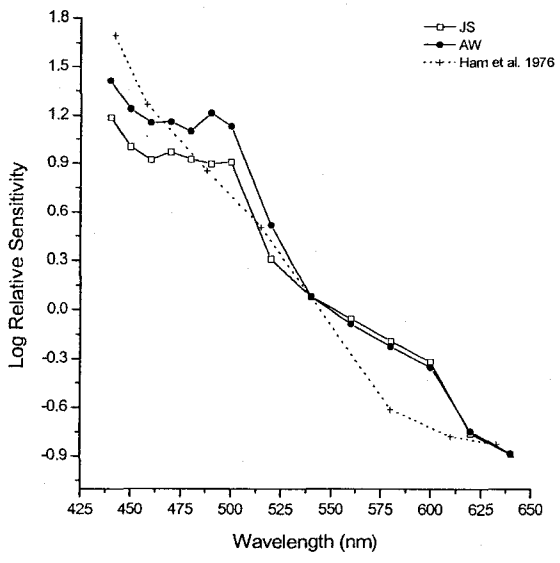

Figure 3: Mean photophobia action spectra for two subjects in Stringham et al's (2003) study, corrected for lens and macular pigment optical density. For comparison, the functions are plotted with the retinal damage function measured by Ham et al (1976).

one can see, for wavelengths between 520nm and 640nm, there was a positive trend between wavelength and the energy needed to produce PP. That is, less energy was needed to induce a squint at 520nm compared to 640nm. At shorter wavelengths the spectra appear as a notch centered at 460nm. Interestingly, the trough and shape of this notch roughly resemble the log transmittance spectrum of MP. In fact, the difference in MPOD between the two subjects seems to account for their slight, but uniform, difference in photophobic sensitivity below 520nm. Further, when the filtering effects of MP, as well as the lens, are accounted for, the notch disappears, and the PP spectra resemble the retinal damage function reported by Ham et al.

(1976; see figure 3). These findings suggest that MP may attenuate PP or discomfort associated with sufficiently intense SW targets and may impact the threshold for PP under normal viewing conditions.

CHAPTER V

RESEARCH OBJECTIVES

The objective of the present project was to test directly the relationship between MP and PP. First, it is hypothesized that these two phenomena are positively correlated such that individuals with higher MPOD will require more energy to experience PP when viewing a SW target relative to a LW target. Second, in an attempt to establish a causal relationship, it is hypothesized that augmenting MPOD via lutein supplementation will result in corresponding increases in the threshold for PP for a SW target relative to a LW target. If this is the case, increasing MPOD, either through diet or supplementation, may represent a viable treatment for some individuals who suffer from frequent episodes of PP.

CHAPTER VI

METHODS

Measurement of Macular Pigment Optical Density

The recent interest in MP's role in the development of AMD has prompted many researchers to devise methods for measuring retinal lutein and zeaxanthin concentrations in vivo. These techniques take advantage of the absorption spectrum and retinal concentration gradient of MP; specifically, the filtration of SW light by the MP in the fovea relative to the parafovea. For example, in fundus photographic techniques (e.g., Kilbride et al., 1989; Abadi and Cox, 1992), two images of a bleached retina are obtained, one using light absorbed by MP and another using light not absorbed by MP. In the former photograph, the MP will appear dark to black. The two photographs are digitized, rendered in grayscale, then aligned using ocular landmarks. MPOD is obtained by subtracting the density differences (grayness) at each pixel between the two photographs. In another technique, a reflection spectrum of the retina is obtained using a scanning laser ophthalmoscope (e.g., Berendschot et al., 2000; Wüstemeyer et al., 2003). This instrument measures the amount of monochromatic light reflected from various retinal loci. To calculate MPOD, the amount of (reflected) energy recorded by a photodetector when the laser falls outside the fovea is compared to the energy when the laser falls on loci in the fovea. Two additional techniques also measure MPOD using reflectometric methods. Bernstein et al. (2002) and Gellerman et al. (2002) measured MPOD by exposing the fovea and parafovea to an argon laser and measuring the distinct

Raman signals of lutein and zeaxanthin. Another innovative technique involves measuring the amount of lipofuscin fluorescence in the fovea and parafovea after exposure to SW and MW monochromatic light (e.g., Delori et al., 2001). Unfortunately, all of the aforementioned techniques require pupillary dilation with mydriatics, and consequently, a lengthy time investment from the subject.

One of the quicker and easier techniques to measure MP, and hence the most popular, involves measuring the effects of MP on spectral sensitivity using a psychophysical technique called heterochromatic flicker photometry (HFP). Like other in vivo measures, this technique takes advantage of the absorption spectrum and retinal concentration gradient of MP; specifically, the filtration of SW light by the MP in the fovea relative to the parafovea. Because the MP absorbs some SW light before it reaches the photopigments, an estimate of MPOD can be obtained by comparing the spectral sensitivity of retinal loci screened by MP with loci where lutein and zeaxanthin do not accumulate. This possibility, however, is predicated on these two retinal loci having identical spectral sensitivities, save the intervention of MP.

The concentration of MP declines exponentially with increasing eccentricity, such that less than 5 percent of the total lutein and zeaxanthin in the retina accumulates beyond 1.6mm ($\approx$ 5 degrees). Consequently, retinal loci five degrees outside the foveal center can be used to obtain a spectral sensitivity relatively unaffected by MP. To compare spectral sensitivities between the foveola and say, a locus 7 degrees outside the fovea, the testing conditions must favor the input of MW and LW cones while eliminating or diminishing the input of SW cones and rods. These actions are necessary because only MW and LW cones remain in relatively equal proportion to one another from the foveola

to 7 degrees eccentricity (Cicerone & Nerger, 1989; Nerger & Cicerone, 1992). The distribution of rods and SW cones, on the other hand, changes dramatically across the macula (Østerberg, 1935; Curcio et al., 1991). One way to diminish the involvement of rods and SW cones in spectral sensitivity measures is to conduct the test using background light that is more strongly absorbed by these receptor types than by MW and LW cones. A background composed of SW light renders the SW cones and rods insensitive to the lights used to measure MPOD. The importance of conducting the test on a SW background is illustrated in figure 4. As one can see, measures of MPOD are relatively unaffected by a broad range of background intensities provided they exceed about 2 log Td, but a barely perceptible background, or no background, results in profound overestimations of MPOD owing to the intrusion of SW cones and rods. Additionally, using a flickering stimulus that alternates at a frequency above the SW-cone fusion threshold can eliminate the contribution of SW cones.

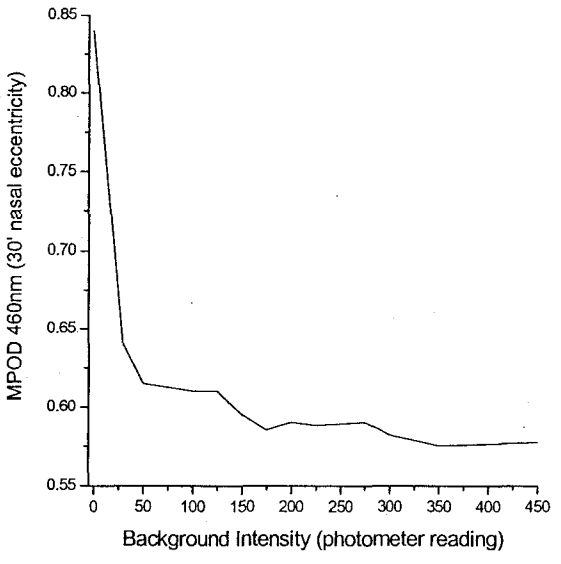

Figure 4: Mean macular pigment optical density (MPOD) as a function of background intensity. The function represents a mean of six sessions for one subject (AW). MPOD was measured in free view at 30′ eccentricity using the Macular Metrics optical system. The flicker frequency of the target was not adjusted during an experimental session.

The stimulus used to measure MPOD with HFP consists of a small flickering disc superimposed on a large, SW background. The flickering disc is composed of two monochromatic lights that alternate in

square-wave counter phase between a reference light and a test light (see figure 5). The radiance and wavelength composition of the reference light remain invariant, while the test light's radiance and wavelength composition are manipulated. To measure MPOD, it is necessary to use a reference light composed of a wavelength outside the absorption spectrum of MP. The chromatic composition of the test light is varied, allowing MPOD estimates at different wavelengths. For each test wavelength, the subject adjusts the radiance of the test light until the test stimulus appears to stop flickering—a condition referred to as sensation luminance (Kaiser, 1988). Measuring the energy necessary for a subject to achieve sensation luminance at various wavelengths yields a spectral sensitivity curve. By obtaining two such spectral sensitivity curves, one in the fovea and another in the parafovea, researchers can determine the effect of MP on spectral sensitivity by normalizing the two functions (see figure 6). A measurement of MPOD can be calculated by taking the log ratio of these two functions. That is, for each test

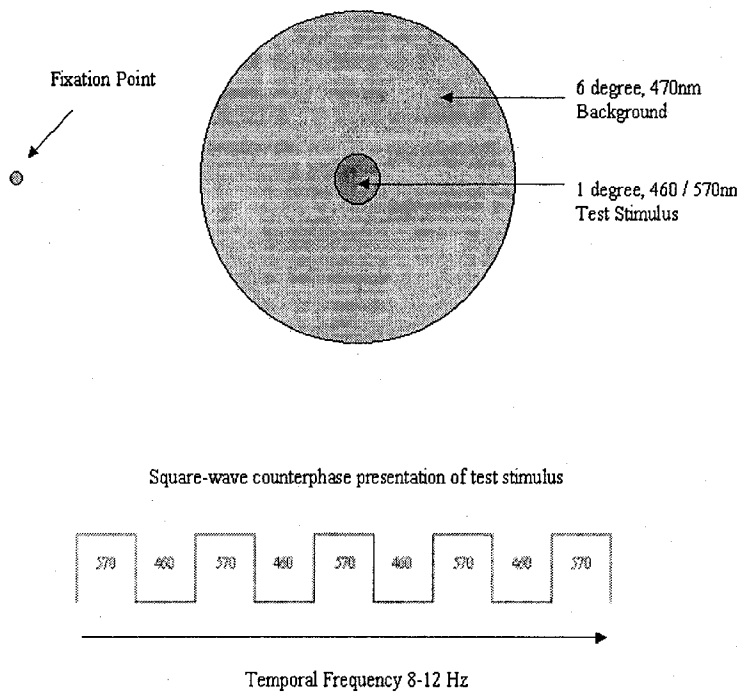

Figure 5: Schematic of the stimulus used to measure macular pigment optical density with heterochromatic flicker photometry. Below the schematic is a graphical representation of the counter-phase presentation of the test stimulus.

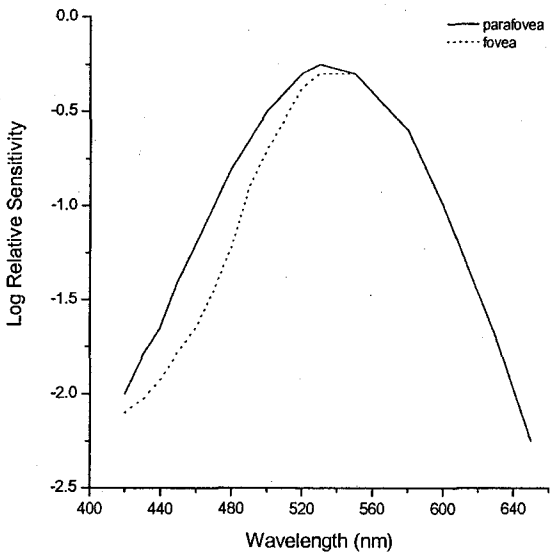

Figure 6: Foveal and parafoveal spectral sensitivity functions measured using heterochromatic flicker photometry. The data are normalized at 560nm. The log ratio of the two functions is attributable to absorption by macular pigment.

wavelength, the log energy necessary to achieve sensation luminance in the fovea minus the log energy needed in the parafovea yields a measure of MPOD. MP absorption spectra obtained using this psychophysical technique are analogous to those obtained via other methods, such as spectrophotometry (Bone et al., 1985), microdensitometry (Snodderly et al., 1984), and fundus reflectometry (Kilbride et al., 1989). To obtain a MP absorption spectrum with HFP requires a lengthy time investment from the subject. As a result, most researchers simply measure MPOD at 460nm, as this is the wavelength of peak absorption. Cautious researchers also occasionally measure MPOD at 490nm as a validity check for a subject's 460nm measure. Measurements using a 490nm test light should yield MPOD approximately half the value obtained using a 460nm test light (Wyszecki & Stiles,1982).

In 1987, Werner et al. measured MPOD after manipulating several parameters of the HFP task. Of particular interest was their finding that MPOD was predicated on the radius of the foveal test stimulus, or more appropriately, its visual angle. They found that as the visual angle of a centrally fixated test stimulus increased, MPOD decreased

exponentially. The relationship between the radius of the test stimulus and MPOD corresponded well with the retinal concentration gradient of MP established with HPLC. This finding suggests that MPOD is predicated on the effects of MP absorption at the edge of a flickering test stimulus. For example, the edge of a centrally fixated one-degree test disc will extend to 0.5 degrees eccentricity, and will yield a measure of sensation luminance affected by MP absorption at this retinal locus. The fact that sensation luminance is dependent on MP absorption at the edge of a flickering disc target, and not on space-averaged MPOD, is further supported by the observation that a one-degree annulus yields the same measure of sensation luminance as a one-degree test disc.

In the current experiment, MPOD was measured at 460nm using four centrally fixated targets—40-minute disc (0.66 degrees), 1-degree disc, 2-degree annulus, 4-degree annulus—and a parafoveal reference target (2 degree disc) centered at 7 degrees of eccentricity, yielding a MPOD profile

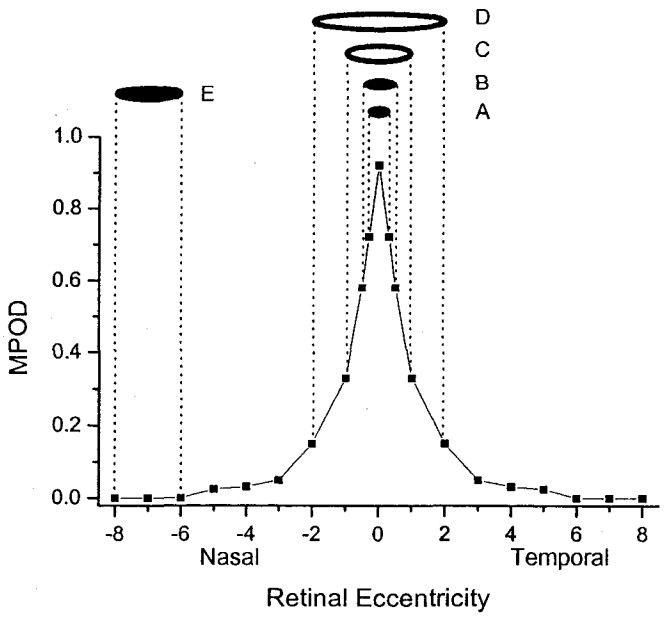

Figure 7: Schematic of the retinal loci and corresponding macular pigment optical density profile assessed using the Macular Metrics densitometer. Stimuli A and B are centrally fixated discs that subtend 40′ and 60′ of visual angle, respectively. Stimuli C and D are centrally fixated annuli, with outer edges subtending 120′ and 240′, respectively. The widths of the annuli subtend 20′ of visual angle. Stimulus E is a 120′ disc centered at seven degrees eccentricity by the subject's fixating a small point of light to the left of the stimulus.

between 20-minutes and 2-degrees eccentricity (see figure 7). The test stimuli were presented in free-view using an optical system recently developed by the Macular Metrics Corporation ® (Rehoboth, MA; see Wooten et al., 1999). This system produces the test stimuli with an arrangement of 4 LEDS—two with a peak wavelength at 458nm, one with a peak at 490nm, and the fourth with a peak at 530nm. The 530nm LED (1.7 log Td) is electronically driven to alternate in square-wave with either the two 458nm LEDs, for estimates of MPOD at its wavelength of peak absorption, or the one 490nm LED for density estimates at this test wavelength. Light from these LEDs is collimated by a planoconvex lens and defined by one of five apertures constructed of Mylar film. Another LED arrangement, comprised of 4 LEDS with a peak spectral emmitance at 470nm is used to produce the background (1.5 log Td). Light from this arrangement passes through a planoconvex lens and is defined by a 6-degree aperture. Finally, light from both LED arrangements is combined using a beam-splitter. When the subject's right eye is properly aligned with the optical axis of the beam splitter, the stimulus appears as a flickering test disc (or annulus) superimposed on a large, SW background (figure 5). At the center of the flickering test stimulus is a small, 5-minutes of arc, fixation point. For the four foveal measures of sensation luminance, the subject fixates this small point as he or she adjusts the intensity of the test LED(s) until flicker is minimized or eliminated. To perform the reference parafoveal measure, the subject fixates a small, LW LED approximately 7 degrees of visual angle from the center of a two-degree test stimulus (disc). This fixation point is located to the left of the test image, resulting in a nasal reference measure of sensation luminance. The subject's task,

however, is identical to the foveal task: to adjust the energy of the test LED(s) until the test stimulus appears stable (i.e., stops flickering).

The ability to stabilize the flickering test stimulus is highly dependent on the flicker frequency of the target, especially in the fovea. If the flicker frequency is too low, the subject will likely be unable to eliminate the apparent flicker of the test target. On the other hand, if the flicker frequency is too high, the test stimulus will appear stable for a broad range of energy (i.e., radiance) supplied to the LED(s). Ideally, the subject should be able to adjust the intensity of the test LED(s) to observe a "zone" of flicker on both sides of a *small* null zone—range of radiances for which the test stimulus appears not to flicker. The subject's specific task is to find this null zone and then estimate its mid point. Typically, a subject's estimate of the mid point for a particular stimulus varies slightly between and within testing sessions. One method to reduce such variability is to adjust the flicker frequency of the test stimulus until the null zone is rather small. In general, the null zone and flicker frequency are related positively, such that increasing the flicker frequency

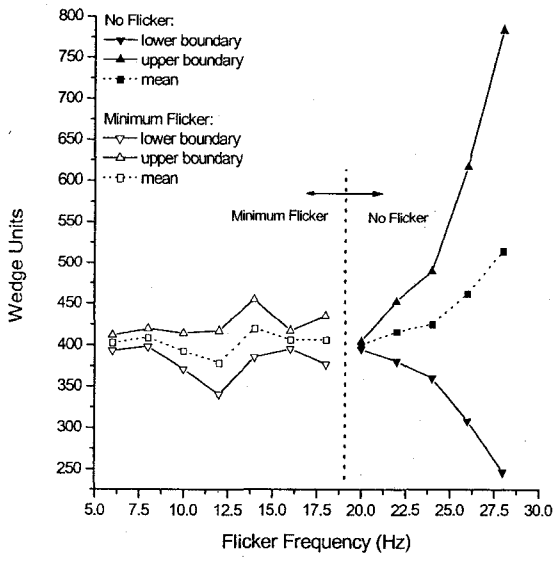

Figure 8: Relationship between flicker frequency of the test stimulus and size of the null zone in HFP for one subject (JMS). The data represent the mean of two sessions and were measured in Maxwellian view with a centrally fixated 40′ target. The spectral composition of the test lights was the same as those used in the current experiment. Above 19Hz, the size of the no-flicker zone increased with increasing flicker frequency. Below 18Hz, the subject could not eliminate flicker.

tends to increase the null zone. The relationship between flicker frequency and null zone size is illustrated in figure 8. In the current project, the flicker frequency of each target was adjusted until the subject perceived a null zone that spanned approximately 100 radiance units according to the Macular Metrics' photometer readout. A null zone of this size is small enough to limit subject variability, but large enough so that subjects can easily and consistently perform estimations of the null zone's mid point. Regardless of the size of the null zone, subjects rarely select the *exact* same mid-point on successive attempts. In order to reduce the effects of such variability, multiple measures are obtained. In fact, data reported by Werner et al. (1987) suggest that the flicker frequency of the target has little effect on calculations of MPOD when several mid-point estimations are obtained for each target. Eight mid-point estimations were obtained for each test stimulus in the current project. The mean radiance of these eight measures was used to calculate MPOD at each eccentricity.

Measurement of Photophobia

Previous researchers investigating the stimulus conditions necessary to induce visual discomfort have failed to control the amount of light entering the eye. Only Stringham et al. (2003) controlled retinal illuminance by presenting their stimuli in Maxwellian view. In this technique, light is focused at the plane of the pupil to a beam narrower than the smallest aperture achievable by the iris, thereby obviating the effects of pupil size on retinal illuminance. This same technique was used in the current experiment. In short, a three-channel Maxwellian view optical system was used to produce the PP test stimuli. One channel was used to produce an 8.2-degree, broadband test stimulus. Light in this channel passed through either a SW broadband filter (Oriel

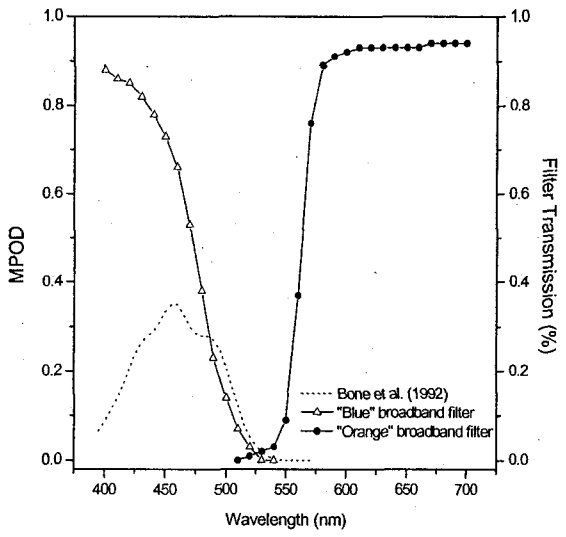

Figure 9: Transmission spectra for the two filters used to produce the stimuli for measures of photophobia thresholds. The light passing through the "blue" broadband filter falls within the absorption spectrum of macular pigment (MP). In contrast, the MP does not absorb any apppreciable wavelengths of light passing through the "orange" broadband filter.

Corp. #59830) or a MW- to LW filter (Tiffen #15). The transmission spectra of these two filters are presented in figure 9. As one can see, the SW broadband filter transmits light strongly absorbed by the MP, whereas the light transmitted by the MW- to LW filter is outside the absorption spectrum of MP. Another channel was used to create a mesopic (-1 log cd/m^2), broadband, 30.5-degree background. The third channel produced a small, 20 minutes of arc, LW fixation light. A schematic of the PP test stimulus is depicted in figure 10. Neutral density filters were used to control the energy in each channel.

At the beginning of each experimental session, the subject's right pupil was aligned with the optical axis of the system using a reticle. Throughout the experiment a dental impression and forehead rests were used by the subject to maintain this position. After the alignment procedure, subjects dark adapted for 20 minutes. Subjects then fixated the small LW light for two minutes. This fixation light was located either centrally, for foveal measures of PP, or at 10.1 degrees temporal eccentricity for parafoveal measures of PP. After the subject adapted to the background for two minutes,

the experimenter announced that the PP stimulus was to be presented. The time between the experimenter's cue and the presentation of the test target was quasi-random, between 5 and 20 seconds. After the five-second presentation of the test target, subjects rated their level of visual discomfort using a 10-point psychophysical scale, where a 1 represented no discomfort and a 10 represented PP—i.e., the light caused sufficient discomfort to elicit a squint and subjects wanted to divert their eye from the target. The initial test target was well below the subject's PP threshold. By use of the method of ascending limits, the energy of the test light was increased by subtracting neutral density from channel one. If the subjects rated their discomfort between 5 and 9, 0.1 log units of density was subtracted, otherwise 0.3 log units was removed from the channel. Random catch trials were performed to assess subject bias. After each presentation of the test stimulus, the experimenter rolled two die. If the sum

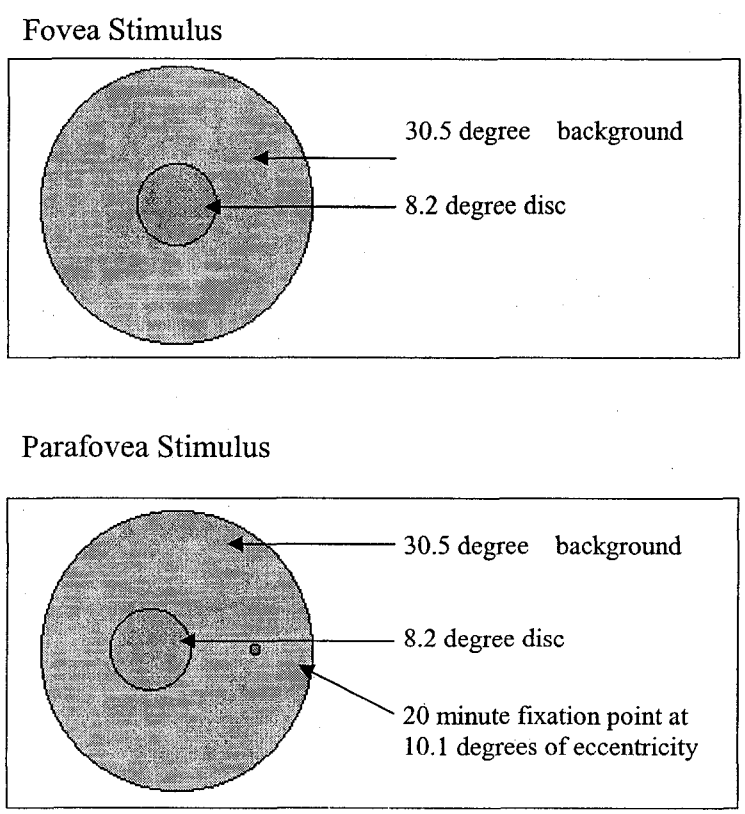

Figure 10: Schematic of the foveal and parafoveal test stimuli used to measure photophobia thresholds. For each retinal loci, the 8.2 degree test disk was composed of light passing through either the broadband "blue" filter or the broadband "orange" filter (see text).

of the die was 2, 0.1 log units of density was added to the test channel. If the subject correctly rated the stimulus as less intense (i.e., a lower number) than the previous target, the response was recorded as a "hit." Conversely, if the subject incorrectly rated the stimulus, rating it as equally or more discomforting than the previous stimulus, it was recorded as a "false alarm" (FA). Additional non-random catch trials were also performed by the experimenter to better define subjects' PP thresholds. To maintain the same level of retinal sensitivity prior to each presentation of the test stimulus, subjects covered their right eye with an eyepatch and dark adapted for fifteen minutes between trials. This procedure was repeated until the subject reported that he or she experienced PP (i.e., a rating of 10) while viewing the test stimulus. PP thresholds for the 8.2 degree test stimulus were measured for the SW ("blue") target in the fovea and parafovea and for the MW- to LW ("orange") target in the fovea and parafovea. The four test conditions are illustrated in figure 11. After the experiment, the energy of the test lights which induced PP were measured using a radiometer (United Detector Technology Optomteter #61). The energy of the four targets was log transformed, and 0.46 log units was subtracted from the log orange energy to correct for the orange filter's greater energy transmission. The corrected log energy of the orange target that induced PP was then subtracted from the log energy of the blue target that induced PP to yield a foveal PP ratio. A parafoveal PP ratio was calculated in the same manner. Finally, a difference ratio (PP ratio) was calculated by subtracting the parafoveal PP ratio from the foveal PP ratio. Before the project was initiated, the above procedure was used to compare PP thresholds measured with a scaling technique to those determined with electromyography (electromyogram—EMG). Most of the studies investigating visual discomfort asked

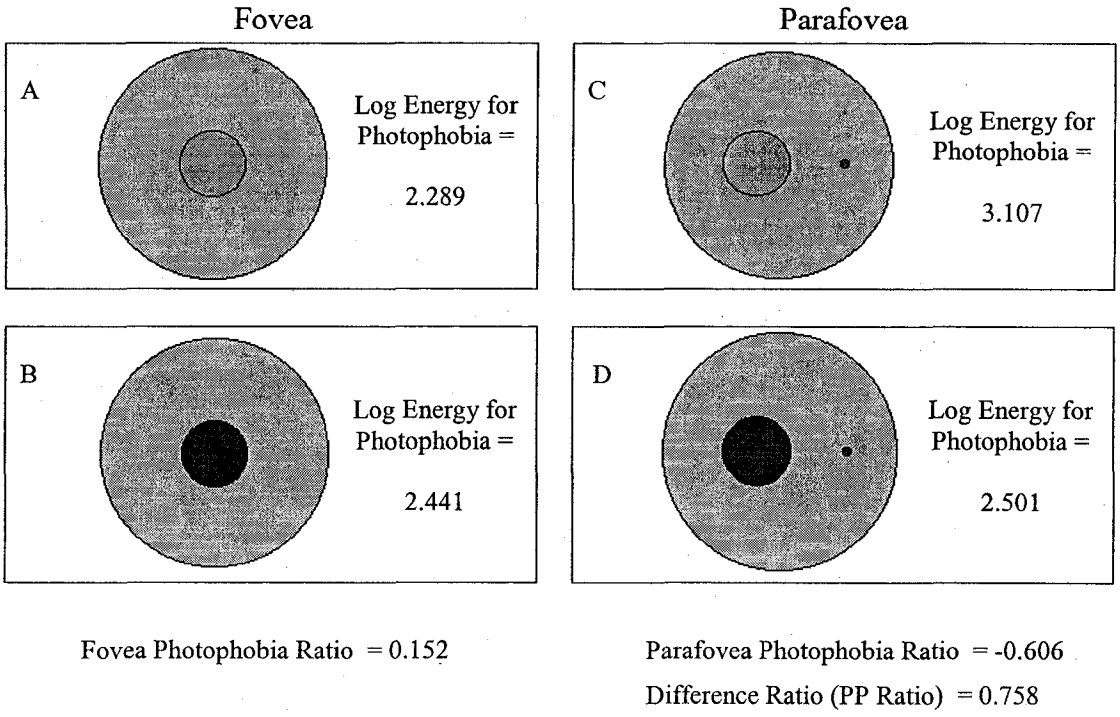

Figure 11: Schematic of the four photophobia (PP) test stimuli used to measure PP thresholds. Stimuli A and B were used to measure foveal PP thresholds for the "orange" and "blue" lights, respectively. Stimuli C and D were used to measure parafoveal PP thresholds for the orange and blue lights, respectively. The calculation of PP ratio is illustrated with an example. In the fovea and parafovea, the log relative energy necessary to induce PP for the orange stimulus was subtracted from the energy necessary to induce PP with the blue stimulus. The absolute difference between these two ratios was called the PP ratio.

subjects to use a scale (as was the case in the present study), such as the de Boer scale (de Boer, 1967), to quantify their level of discomfort when viewing a light source. As mentioned earlier, two groups of researchers, however, attempted to measure PP using an "objective" procedure. Both Berman et al. (1994) and Stringham et al. (2003) recorded gross muscle potentials around the eye (EMG) while their subjects viewed a light source. Essentially, these researchers assumed that a sufficiently bright light will compel a subject to squint in order to limit the amount of light entering the eye. When the signal-to-noise ratio of the EMG trace (i.e., squint magnitude) exceeded a criterion value, the subject was defined operationally to have experienced PP. Berman et al. (1994) and

Stringham et al. (2003) also asked their subjects to rate their level of discomfort using a scale. Interestingly, both groups of researchers noted a high degree of correspondence between squint magnitude and the subjects' subjective rating of discomfort. In fact, Stringham et al. showed that the PP action spectrum obtained using EMG looked essentially identical to the spectrum based on their subjects' subjective ratings of lights just below the PP threshold. Their subjects used the same10-point psychophysical scale used in the present study, where 1 signified no discomfort and a 10 represented PP.

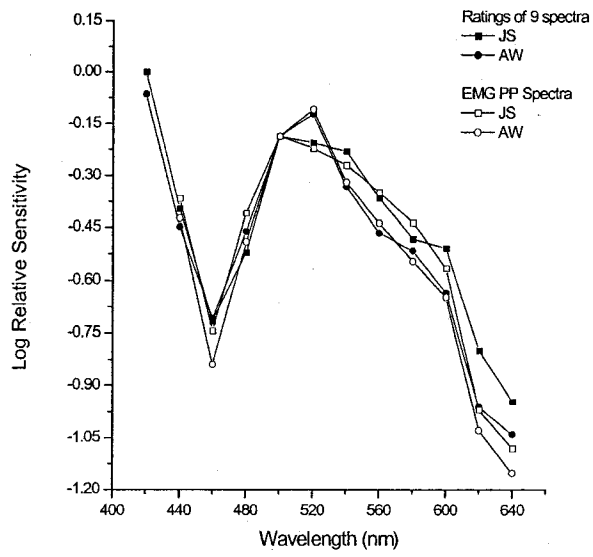

Figure 12: Mean electromyographic and scaling photophobia action spectra for two subjects in Stringham et al's (2003) study. The functions are normalized at 500nm.

Figure 12 shows their subjects' action spectra for subjective ratings of "9," which represent extreme discomfort, bordering on PP, compared to the EMG-determined spectra of PP. The similarity between the scaling and EMG functions suggests that a rating scale is a viable option for estimating PP, particularly if subjects are well trained and PP is well defined.

In the studies by Berman et al. and Stringham et al., the high degree of correspondence between the objective (EMG) and subjective (scale) values may have been partly due to the fact that the scaling procedures were coincident with the EMG recordings. To obviate this possibility, one naïve subject and one well trained subject had their PP thresholds for the blue and orange

targets measured in two separate sessions. In one session, PP thresholds were measured using the aforementioned techniques (i.e., subjects used a psychophysical scale). In the other session, PP thresholds for the same four targets were determined using EMG. Three nickel-plated, surface electrodes were used to record gross potentials generated during a squint: one electrode was attached below the subject's right eye (test), another on the subject's right temple (reference), and a third on the back of the subject's neck (ground). The electrodes were connected to a Grass Instruments amplifier (#7P3B), which sent the signal to a waveform computer program. As in Stringham et al., PP was defined as a signal-to-noise ratio of 4:1 on the EMG trace lasting at least 2.5 seconds. The data for both subjects' sessions are presented in figure 13. The high degree of similarity between the two sessions, particularly JMS's functions, suggests that a scaling technique can be reliably used to determine PP thresholds. Further, a psychophysical scaling procedure has two primary advantages over EMG for determining PP thresholds. It is less invasive for the subject; and more importantly, EMG can only be used to measure squint magnitude (behavior), not visual discomfort (PP).

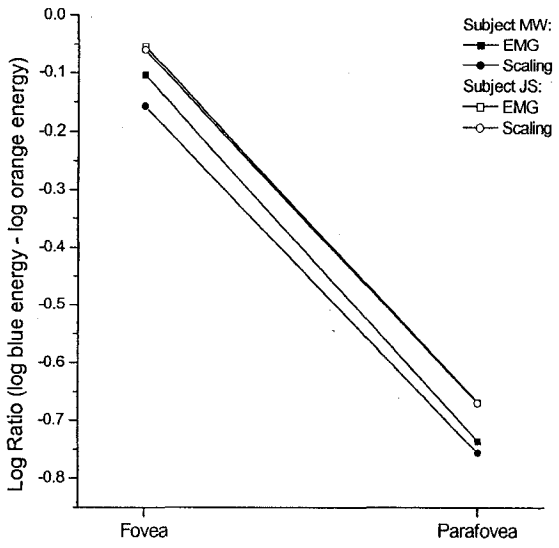

Figure 13: Foveal and parafoveal photophobia ratios for two subjects measured with electromyography and a scaling technique. The data are not normalized.

Experimental Protocol

To adequately investigate the impact of MPOD on PP, two separate experiments were performed. The primary goal of the first experiment was to investigate the correlation between MPOD and the energy necessary to induce PP for the blue target relative to the orange target. Using the aforementioned techniques, MPOD and PP thresholds were measured in ten, non-smoking individuals, four males and six females, ages 21 to 33.

The primary goal of the second experiment was to measure the effects of augmented MPOD on the energy needed to induce PP for the four targets. Four subjects from experiment one, two males and two females, ages 24 to 31, took 60mg of Xangold™ (Cognis, La Grange, IL) dietary supplements daily for twelve weeks. The dosage was equal to approximately 30mg of free lutein per day. During the twelve weeks of intervention, subjects continued their regular diet. PP thresholds and MPOD were measured at baseline and again after six weeks and twelve weeks of supplementation.

The use of human subjects in this project was approved by the University of New Hampshire Institutional Review Board.

CHAPTER VII

RESULTS[1]

Experiment One

The sample's mean age, BMI, and MPOD at each eccentricity are presented in table 1 (see Apendix A). BMI was linearly correlated with MPOD at 60′ eccentricity ($r = 0.786$, $n = 10$, $p = 0.006$), although this relationship was largely driven by two outliers. Age, on the other hand, was not significantly correlated with MPOD at any eccentricity.

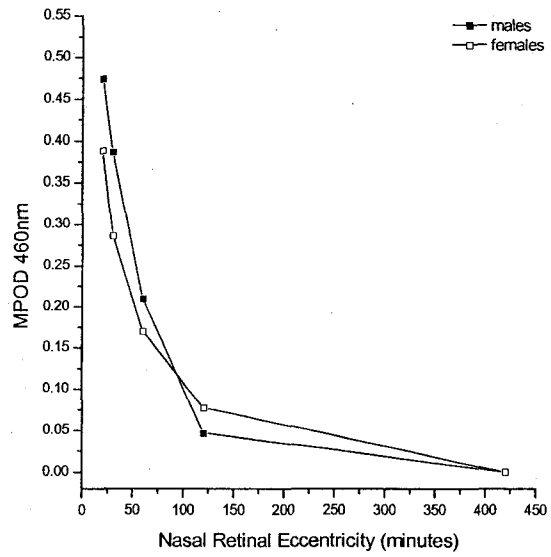

Figure 14: Mean male (n = 4) and female (n = 6) macular pigment optical density (MPOD) profiles. MPOD did not significantly differ between males and females at any loci.

The mean MPOD profiles for males and females are illustrated in figure 14. Although the sample mean male MPOD profile is higher at the three central loci, and the female sample mean at 120′ eccentricity, the differences were not statistically significant according to independent sample t-tests. Likewise, there was no significance difference in BMI or age between males and females.

[1] The familywise error rate (i.e., probability of making one or more type I errors) for all planned contrasts is 0.641.

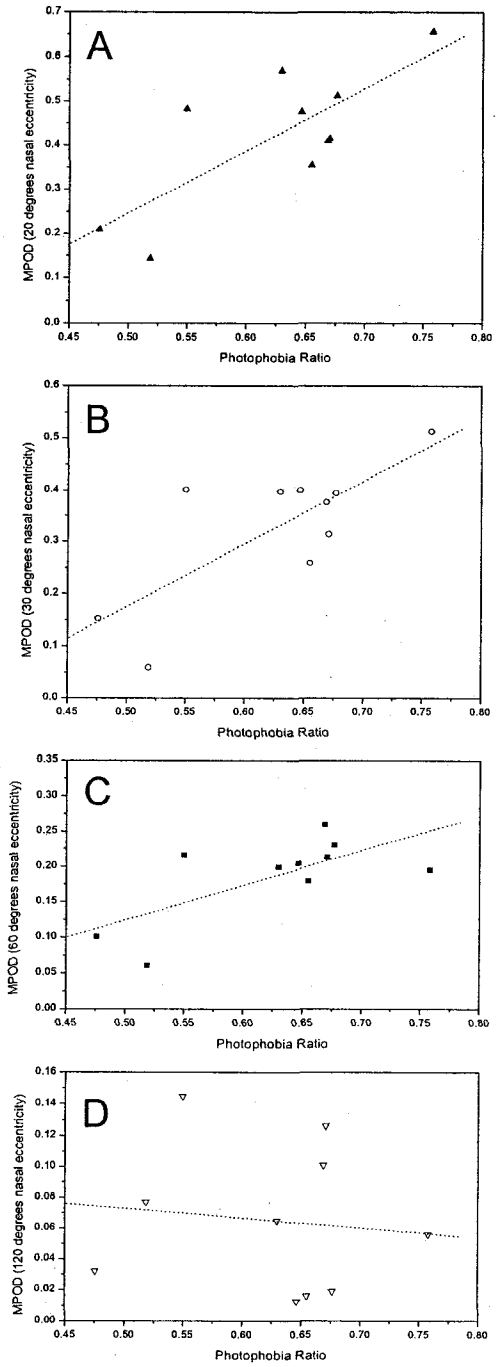

Figure 15: Relationship between PP ratio and MPOD. There was a significantly linear relationship between PP ratio and MPOD at 20′ (A, p = 0.009), 30′ (B, p = 0.010), and 60′ (C, p = 0.025) eccentricity. MPOD at 120′ (D, p = 0.750) eccentricity was not linearly related to PP ratio.

The ten subjects' MPOD at each eccentricity and PP ratios were plotted and fit with a linear function. As shown in figure 15a-d, there was a significant linear relationship between the subjects' PP ratios and MPOD at eccentricities of 20′ ($r = 0.767$, $p = 0.009$), 30′ ($r = 0.760$, $p = 0.010$) and 60′ ($r = 0.694$, $p = 0.025$), and a non-significant relationship between PP ratio and MPOD at 120′ eccentricity ($r = -0.115$, $p = 0.750$).

In the event that PP thresholds for SW lights are affected by the aggregate filtration of MP in the fovea, an estimate of total screening was calculated by taking the area under the MPOD profile. Each subject's MPOD profile was plotted and fit with a Gaussian function, using Origin

7.0® (Northampton, MA). The area under the MPOD profile was calculated to yield a Gaussian integrated MPOD (iGMPOD). Several previous researchers have used the Gaussian function to explain the foveal distribution of MP (e.g., Snodderly et al., 2004).

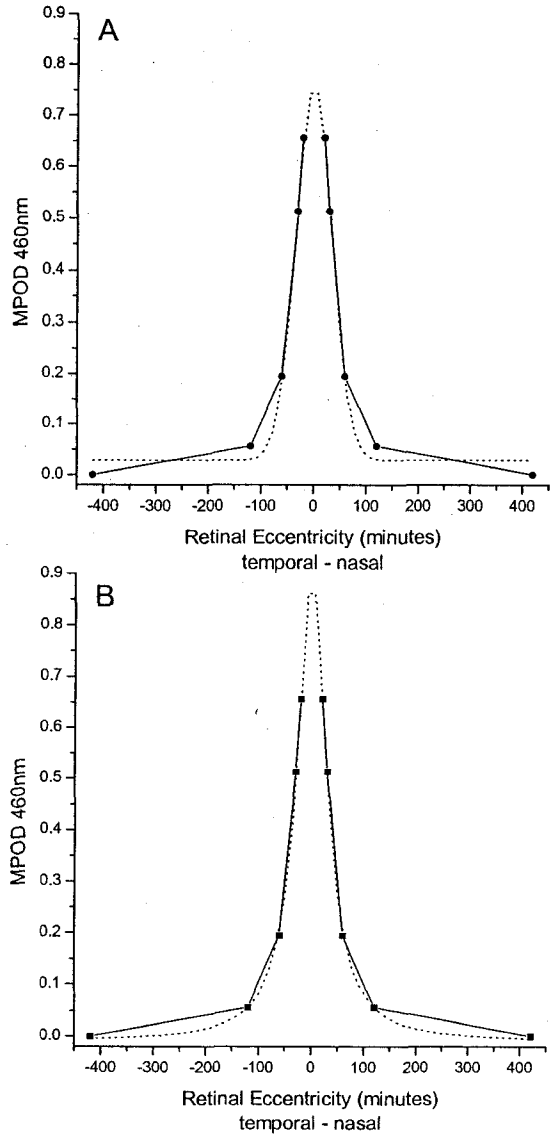

Figure 16: Subject AW's macular pigment optical density profiles fit with a Gaussian function (A) and a Lorentzian function (B).

The Gassian function can be described with the following formula:

$$y = y_0 + \frac{A}{w \cdot \sqrt{\frac{\pi}{2}}} e^{-\frac{2(x - x_0)^2}{w^2}}$$

where y_0 = baseline offset (i.e., ordinate value of the curve's asymptote); A = total area under the curve from the baseline; X_0 = center of the peak (i.e., mean); w = width of the peak at half height. Figure 16a shows subject AW's MPOD profile fit with a Gaussian function. The Gaussian function appears to describe the MPOD profile well, with an $R^2 = 0.994$. For the other nine subjects, the R^2 for the Gaussian fit ranged from 0.999 to 0.817. The iGMPOD and PP ratios

for each subject are plotted in figure 17a. As one can see, there was a strong positive relationship between iGMPOD and PP ratio ($r = 0.830$, $n = 10$, $p = 0.002$).

The fovea distribution of MP has also been estimated using the Lorentzian distribution (e.g., Stringham et al., 2003). The Lorentzian distribution can be described by the following formula:

$$y = y_0 + \frac{2 \cdot A}{\pi} \cdot \frac{w}{4(x - x_0)^2 + w^2}$$

where y_0 = baseline offset, A = total area under the curve from the baseline; X_0 = center of the peak; and w = width of the peak at half height. Figure 16b shows subject AW's MPOD profile fit with a Lorentzian function. For subject AW, the Lorentzian distribution appears to "fit" his MPOD profile slightly better than the Gaussian distribution, with an R^2 of 0.999. Except for two subjects, the Lorentzian distribution fit each subject's MPOD profile slightly better (i.e., higher R^2) than did the

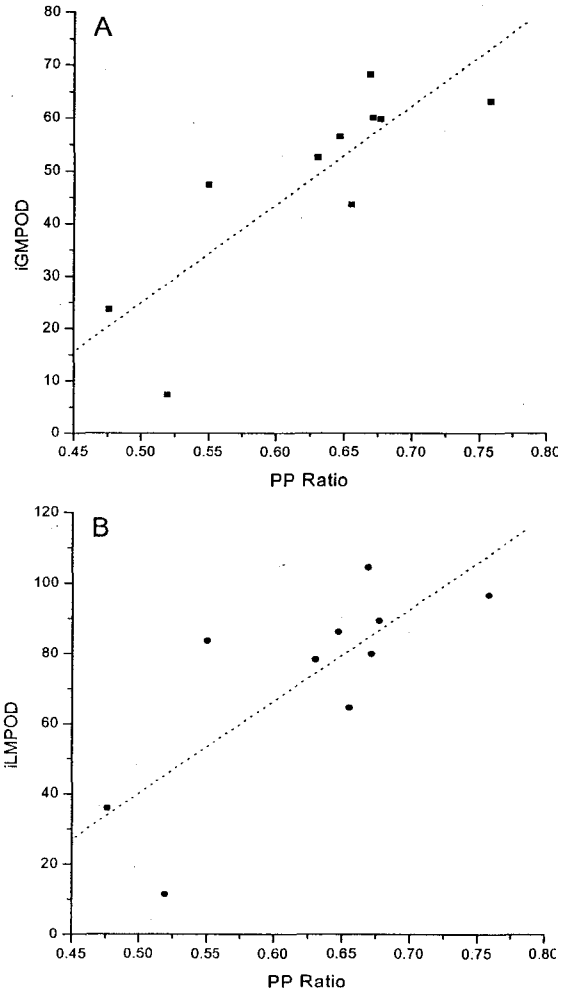

Figure 17: Relationship between integrated macular pigment optical density (iMPOD) and photophobia ratio (PP ratio). The top panel (A) shows the significant, positive relationship between integrated Gaussian MPOD and PP ratio ($p = 0.002$). The bottom panel (B) shows the significant linear relationship between integrated Lorentzian MPOD and PP ratio ($p = 0.008$).

Gaussian function. For one subject, the Gaussian distribution fit his MPOD profile better, and for another subject, who had virtually no MP, the Gaussian distribution and Lorentzian distribution had the same coefficient of determination ($R^2 = 0.810$). The area under the Lorentzian fit of each subject's MPOD profile was calculated to estimate an integrated Lorentzian MPOD (iLMPOD). The relationship between iLMPOD and PP ratio is shown in figure 17b. Like iGMPOD, iLMPOD was positively related to PP ratio ($r = 0.775$, $n = 10$, $p = 0.008$).

To properly fit each subject's MPOD profile with the Gaussian and Lorentzian functions, it was necessary to estimate their temporal MPOD profile. Previous research has demonstrated that MP is distributed symmetrically about the fovea (Hammond et al., 1997b), such that MPOD at 30′ eccentricity in the nasal retina is nearly identical to MPOD at 30′ in the temporal retina. Consequently, an estimate of MPOD in the temporal retina may be obtained by taking the MPOD measured at a corresponding locus in the nasal retina. This possibility was tested by measuring a detailed nasal and temporal MPOD profile in two subjects. The profiles were measured using the Macular Metrics optical system mentioned above, with one modification: additional fixation points were added to measure sensation luminance at 150′ and 300′ eccentricity in the nasal and temporal retina, and seven-degrees in the temporal retina. As shown in figure 18a-b, there was a high degree of uniformity between the subjects' nasal and temporal MPOD profiles. In fact, for subject JMS, the two profiles match almost perfectly. The iGMPOD using both profiles is 41.63 for JMS and 69.89 for AW. When iGMPOD is calculated using the nasal profile and an estimated temporal profile (as was done in the calculations above), iGMPOD for JMS is 41.75, and for AW, 67.33. The iLMPOD for JMS, using

both the nasal and temporal profiles, was 60.27, and for subject AW, 103.28. Calculating iLMPOD using the nasal profile and an estimated temporal profile yielded an integrated area of 60.54 for JMS, and 99.43 for AW. Thus, using the nasal profile to estimate MPOD in the temporal retina resulted in a mean underestimation of integrated area of less than two-percent. Estimates of the MPOD peak derived using the Gaussian or Lorentzian functions differed less than one-percent between the values obtained using both profiles and the values obtained using the nasal profile and an estimated temporal profile. These results suggest that a Gaussian or Lorentzian integrated MPOD and peak optical density can be estimated accurately by measuring MPOD in the nasal retina and using these values as a measure of MPOD in the temporal retina.

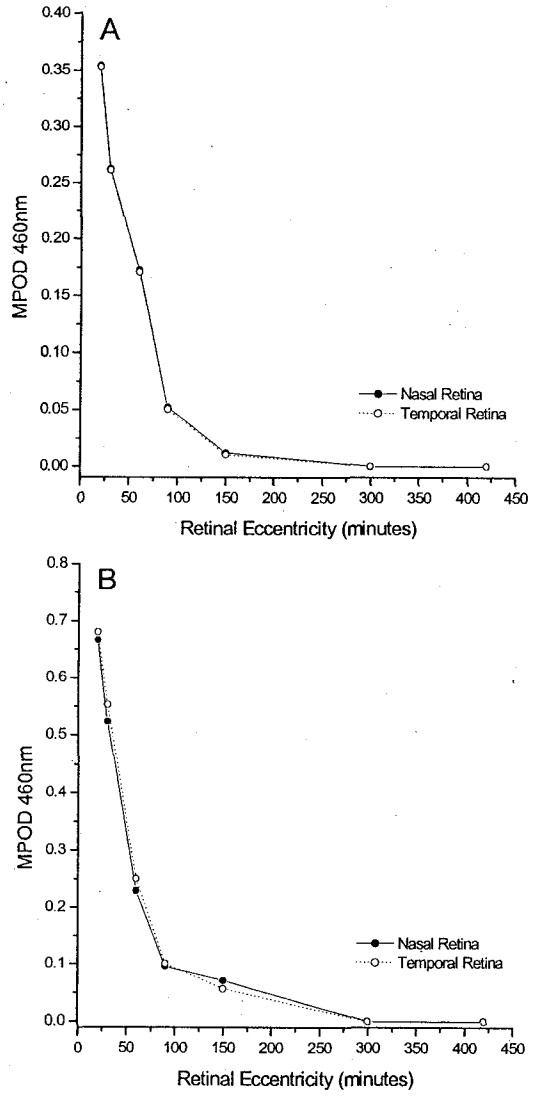

Figure 18: Nasal and temporal macular pigment optical density (MPOD) profiles for subjects JMS (A) and AW (B). The profiles are normalized at 420′ eccentricity.

Experiment Two

The four subjects' MPOD profiles at baseline and after six and twelve weeks of lutein intervention are depicted in figure 19a-d. The group's mean MPOD profile at baseline, visit two and visit three is shown in figure 20. The figure shows that mean MPOD increased slightly at the central three loci after six weeks of intervention by the consumption of approximately 1,260mg of lutein (30mg per day). After an additional six weeks of lutein intervention, mean MPOD increased another 0.04 log units at the two central loci and about 0.02 log units at the other two loci. According to a randomized

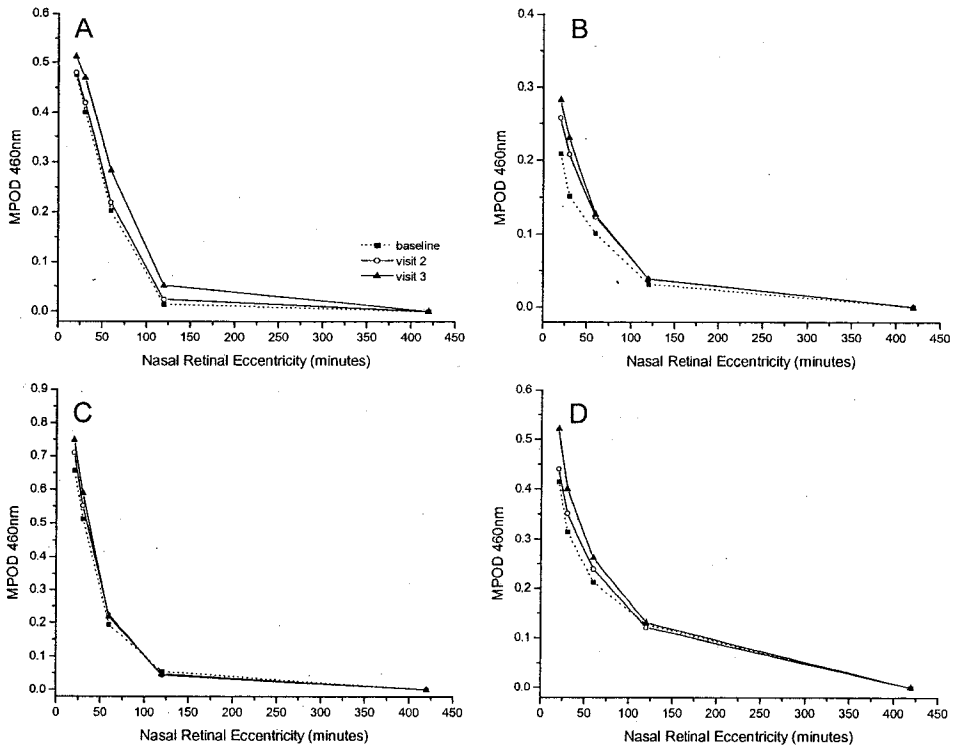

Figure 19: Macular pigment optical density profiles for the four subjects in Experiment Two at baseline and after six-weeks (visit 2) and twelve-weeks (visit 3) of lutein intervention. The MPOD profiles are normalized at 420′ eccentricity. Panels A, B, C, and D show the MPOD profiles for subjects JPS, LS, AW and MW, respectively.

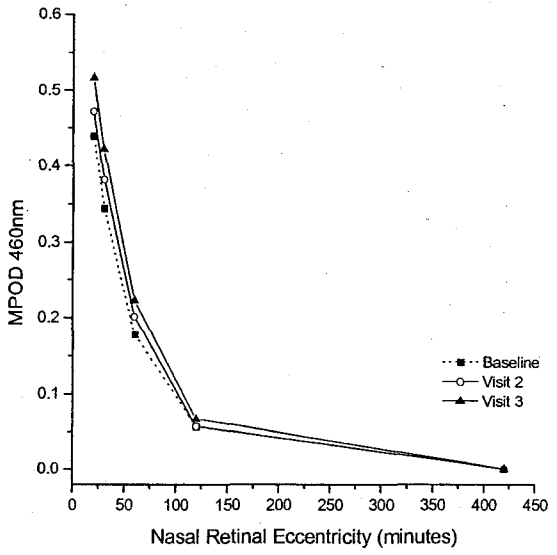

Figure 20: Mean macular pigment optical density profiles for the subjects in Experiment Two at baseline and after six-weeks (visit 2) and twelve-weeks (visit 3) of lutein intervention. According to a subjects-by-trials analysis, MPOD significantly increased from baseline at 20′ ($p = 0.003$), 30′ ($p < 0.001$), and 60′ ($p = 0.026$) eccentricity.

block design (RB-3; Kirk, 1995; pages 251-286), MPOD significantly increased from baseline at 20′ ($F_{2,6} = 17.35$, $p = 0.003$, $\omega^2 = 0.73$), 30′ ($F_{2,6} = 77.00$, $p < 0.001$, $\omega^2 = 0.92$) and 60′ ($F_{2,6} = 7.04$, $p = 0.026$, $\omega^2 = 0.50$). The changes in MPOD at these three loci after six and twelve weeks of lutein intervention can be described by a linear trend (20′: $F = 26.04$, $p = 0.014$, $\omega^2 = 0.89$; 30′: $F = 495.70$, $p < 0.001$, $\omega^2 = 0.93$; 60′: $F = 11.29$, $p = 0.043$, $\omega^2 = 0.74$), according to analyses using orthogonal polynomial contrasts. Changes in MPOD did not depart from linearity.

An integrated MPOD was calculated for each subject's three MPOD profiles using the aforementioned Gaussian and Lorentzian distributions. The Lorentzian distribution fit the data slightly better than did the Gaussian function for three of the four subjects, using R^2 as a measure of "goodness of fit." For the Gaussian fit , R^2 ranged from 0.999 to 0.950, and for the Lorentzian fit, it ranged from 0.999 to 0.984. Mean iGMPOD increased from 50.86 (SD = 18.30) at baseline to 56.10 (SD = 17.88) after six weeks of intervention, and to 60.27 (SD = 19.48) after twelve weeks. Mean iLMPOD increased from 74.69 (SD = 26.78) at baseline to 84.27 (SD = 25.55) after six weeks of

intervention, and to 92.63 (SD = 29.41) after twelve weeks. Thus, after six weeks of intervention, the area under the mean MPOD profiles increased approximately 11 percent, and after twelve weeks, approximately 22 percent. Both iGMPOD ($F_{2,6} = 9.12$, p = 0.015, $\omega^2 = 0.57$) and iLMPOD ($F_{2,6} = 17.35$, p 0.003, $\omega^2 = 0.73$) significantly changed from baseline, according to analyses using an RB-3 design. A linear trend described the changes in iGMPOD (F = 16.74, p = 0.026, $\omega^2 = 0.75$) and iLMPOD (F = 39.75, p = 0.008, $\omega^2 = 0.83$), with no departures from linearity.

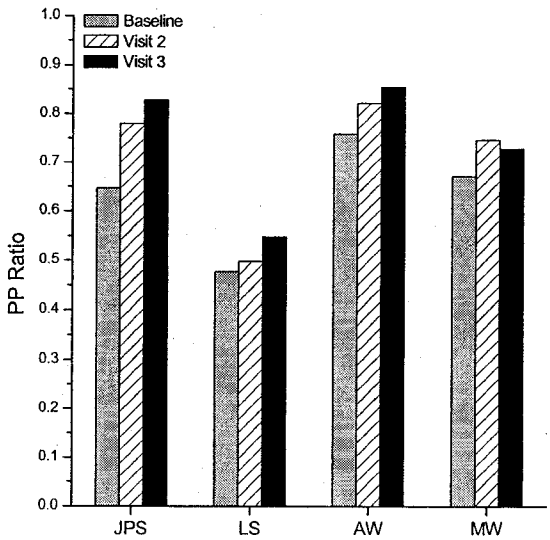

Figure 21: Photophobia ratios (PP ratio) for the four subjects in Experiment Two at baseline and after six-weeks (visit 2) and twelve-weeks (visit 3) of lutein intervention. The subjects' mean PP ratio significantly increased from baseline (p = 0.011) according to a subjects-by-trials design.

The PP ratios for each subject increased after six weeks of intervention, and in three subjects, increased from visit two to visit three (see figure 21). A subjects-by-trial (RB-3) design revealed that PP ratios significantly changed after twelve weeks of lutein intervention ($F_{2,6} = 10.41$, p = 0.011, $\omega^2 = 0.61$). As was the case for MPOD, a linear trend appeared to describe the changes in PP ratio (F = 13.09, p = 0.036, $\omega^2 = 0.86$).

Figure 22a-b shows the ten PP ratios and corresponding iGMPOD (a) and iLMPOD (b) from Experiment One, as well as the additional eight data points from

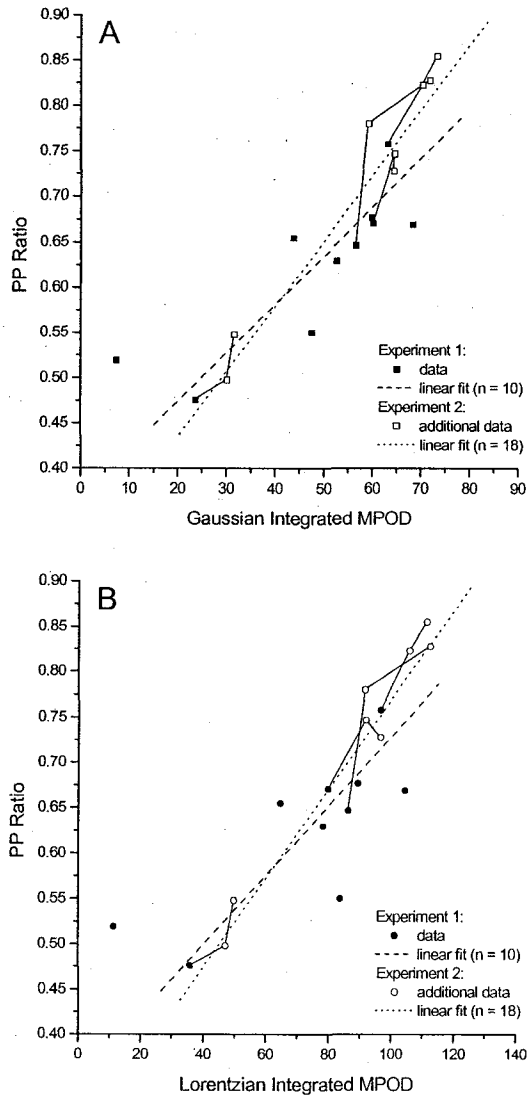

Figure 22: The combined Gaussian (A) and Lorentzian (B) integrated macular pigment optical densities (MPOD) and photophobia ratios (PP ratio) observed in Experiment One and Experiment Two. Two linear fits are plotted in each panel. One linear fit shows the relationship observed in Experiment One (solid line), whereas the second shows the relationship between integrated MPOD and PP ratio for the combined data of both experiments (dotted line). The repeat measures for the four subjects in Experiment Two are connected with solid lines.

Experiment Two. The linear fit from Experiment One and a linear fit of the data from both Experiments (n = 18) is plotted in the figures. The figure shows that the inclusion of data from Experiment Two has a slight impact on the linear fits. In both instances, there is a modest change in slope. If the subject with low MPOD is removed from the analyses, the linear fits of Experiment One (n = 9), and Experiment One plus Experiment Two (n = 17), are nearly identical, as shown in figure 23a-b.

Twenty-six random catch trials were performed while measuring PP ratios in both Experiments. An additional seventeen non-random catch trials were used by the Experimenter to better define subjects' PP

thresholds. The random catch trials had a hit:FA ratio of 12:1 and the non-random catch trials had a hit:FA ratio of 7.5:1. Catch trials were not used in previous investigations of PP; as a result, an acceptable hit:FA ratio has not been determined. However, the hit:FA ratio observed in the current study was sufficiently high to suggest that PP thresholds can be measured reliably using the method of ascending limits and a scaling technique.

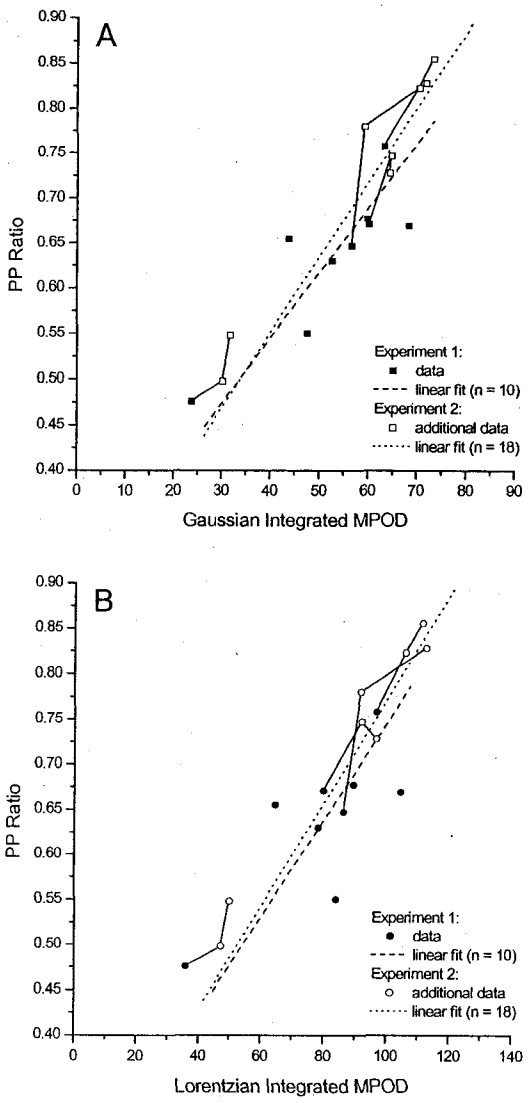

Figure 23: Linear relationships between photophobia ratio and integrated Gaussian (A) and Lorentzian (B) macular pigment optical density after removing subject CF's data. Both panels contain linear fits of the data from Experiment One (solid line), as well as a linear fit of the combined data from both Experiments (dotted line). The repeat measures for the four subjects in Experiment Two are connected with solid lines.

CHAPTER VIII

DISCUSSION

The main finding of this project was that MPOD was associated with PP thresholds for a SW target relative to a MW target. In Experiment One, MPOD at three foveal loci was positively correlated with PP ratios, as was Gaussian and Lorentzian integrated MPOD. In Experiment Two, significant increases in MPOD after twelve weeks of lutein supplementation resulted in significant increases in PP ratios.

Distribution of Macular Pigment

The MPOD of the subjects in Experiment One is similar to previous research conducted using HFP. In several studies, Hammond et al. (1995; 1996a, b, c; 1997b) reported that MPOD at 30′ eccentricity ranged between 0.10 and 0.57. For the small sample in Experiment One, MPOD ranged between 0.06 and 0.51 at 30′ eccentricity. One consideration in comparing the results of the current project to those of Hammond and colleagues involves the spectral bandwidth of the test lights. In the studies by Hammond et al., the half-bandwidth of the test light was 8nm, whereas the half-bandwidth of the test light in the current project was 20nm. The broader spectral emission of test light used in the current study will result in a greater underestimation of MPOD at 460nm than the one used by Hammond and colleagues. Although it is negligible, another consideration is the peak wavelength of the test lights used by Hammond et al. In their studies, the peak wavelength of the test light was 460nm, the absorption peak of MP, whereas the test light in the Macular Metrics system has a peak

wavelength of 458nm, 99.8% of the MP absorption peak. Regardless of the half-bandwidth or peak wavelength of the test lights, the mean MPOD at 30′ eccentricity for subjects in Experiment One is considerably higher than the mean MPOD reported in larger studies (n > 200) that used the Macular Metrics optical system. In a Midwest sample, Cuilla et al. (2001a) found that the mean MPOD at 30′ was 0.211 (SD = 0.13). Similarly, the mean MPOD at 30′ eccentricity in Hammond and Caruso-Avery's Southwest sample was 0.22 (SD = 0.13). One possible explanation for the difference between these mean values and the values in this study is the retinal eccentricity of the parafoveal measure. In the current project, a sensation luminance unaffected by MP (reference) was obtained at six-degrees eccentricity. Cuilla et al. and Hammond and Caruso-Avery used a reference point of four-degrees eccentricity. If MP is present at four-degrees eccentricity, and some research supports this possibility (e.g., Robson et al., 2003), a reference measure at this locus will be a spurious measure of sensation luminance unaffected by MP. In other words, subjects' MPOD at 30′ eccentricity would be underestimated by their amount of MPOD at four-degrees. The spectral composition of the test target and eccentricity of the reference measure are just two factors that make between-study comparisons difficult. The ability to compare results between studies is further complicated by the manifold techniques used to measure MPOD in vivo. However, the range and mean MPOD in the current sample agrees well with values reported using other techniques and optical systems for nearly the same retinal loci (e.g., Kilbride et al., 1989; Beatty et al., 2001; Robson et al., 2003).

Few researchers have used HFP to measure MPOD across the fovea. One of the first was Werner et al. (1987), who measured MPOD at six retinal loci in one observer to

show that measures of MPOD using centrally fixated targets are predicated on absorption at the edge of the test target. The majority of studies investigating the foveal distribution of MP used reflectometry techniques. A general finding across these studies is a high degree of similarity between the nasal and temporal MP profiles, as well as the inferior and superior MP profiles. This relationship, or symmetry, has also been noted in studies using HFP (Hammond et al., 1997b). The symmetry and distribution of MP across the fovea is often described using a Gaussian function. For example, Chen et al. (2001) found that the Gaussian function provided a good fit (mean $R^2 = 0.946$, $n = 54$) for the MP profiles they obtained using fundus reflectometry. Hammond et al. (1997b) reported a mean R^2 of 0.82 ($n = 32$) for the Gaussian fits of their subjects' MPOD profiles. One indirect finding of the current study was that the Lorentzian distribution may provide a better estimate of the foveal distribution of MP compared to the commonly used Gaussian distribution. The mean Gaussian R^2 for subjects in Experiment One was 0.957. For the Lorentzian fit, the mean coefficient of determination was 0.968. If the subject with extremely low MPOD (and an uncharacteristic profile) is removed, the mean Gaussian R^2 is 0.973, and the mean Lorentzian R^2 is 0.986. In both cases, the mean Gaussian coefficient of determination is higher than those reported by Chen et al. (2001) and Hammond et al. (1997b). This finding may be the result of chance or reflect reliable subject performance in the current study. Regardless, the mean Lorentzian R^2 is greater than the Gaussian coefficient of determination, and the Lorentzian distribution provided a better fit for eight of the ten subjects' MPOD profiles in Experiment One. This suggests that the Lorentzian distribution may provide a better model of MP across the fovea.

The primary limitation of using HFP to measure MPOD profiles is the inability to measure MPOD at zero degrees eccentricity. To do so would require infinitesimally small centrally fixated targets. The smallest targets that can be used, however, that will allow for reliable results are on the order of 12′ of visual angle. Thus, one benefit of fitting a MPOD profile with a Gaussian or Lorentzian distribution is the ability to estimate a peak MPOD. A measure of MPOD close to zero degrees was obtained by Hammond et al. (1997b). They measured MPOD using a centrally fixated 12′ target and referred to this measure as peak MPOD, although it was technically a measure of MPOD at 6′ eccentricity. They found that MPOD at 6′ is approximately 39.6% higher than MPOD at 30′ eccentricity. The authors also commented that a Gaussian fit frequently underestimated this peak. Chen et al. (2001) made the same observation. The mean estimated Gaussian peak (6′ eccentricity) of subjects in Experiment One was approximately 27.8% higher than their mean MPOD at 30′ eccentricity. Given the relationship between MPOD at 6′ and 30′ eccentricity observed by Hammond et al. (1997b), it appears that the Gaussian fit also underestimated peak MPOD in the current project. However, the Lorentzian estimated peak of subjects in Experiment One was 34.7% higher than their MPOD at 30′ eccentricity. This suggests that in addition to providing a better fit of the MPOD profile, the Lorentzian distribution may provide a better estimate of peak MPOD than does the Gaussian function.

Lutein Intervention and Macular Pigment Optical Density[2]

Since lutein and zeaxanthin were identified as the primary constituents of MP (Bone et al., 1985), numerous researchers have investigated the effects of lutein

[2] Table 2 (Appendix B) summarizes the various interventions studies described in this section.

intervention on MPOD. For example, Hammond et al. (1997a) measured MPOD at 30′ eccentricity during a fifteen-week intervention in which subjects consumed 60g of spinach and 150g of corn daily. The daily combined dose of lutein was approximately 11.2mg, and the combined dose of zeaxanthin was approximately 0.6mg. The intervention provided about four to ten times as much lutein and zeaxanthin as an average diet (Brady et al., 1996; Tucker et al., 1999; Rock et al. 2002). After four weeks of intervention, mean MPOD significantly differed from baseline, increasing from 0.39 to 0.50. In the current project, mean MPOD at 30′ after twelve weeks of intervention increased from 0.34 to 0.42, slightly less than the mean change reported by Hammond et al. The increase in MPOD observed by Hammond et al. (1997a) appeared to plateau after four weeks of intervention. In contrast, MPOD in the current project increased linearly from baseline, such that the mean increase in MPOD after six weeks of intervention (0.04) was approximately half the increase observed at twelve weeks. Further, all four subjects consuming lutein appeared to respond to the intervention. Three of the ten subjects consuming spinach and corn in Hammond et al.'s study failed to respond to the increased consumption of lutein and zeaxanthin.

In one "responder"—an individual who shows increases in MPOD as a result of increased intake of lutein—Hammond et al. (1997a) measured a detailed MPOD profile at baseline and again after 12 twelve weeks of dietary modification. Changes in MPOD were not uniform across the central retina. Instead, MPOD increased more in central loci than in peripheral loci. In this subject, MPOD increased 0.49 log units at 30′ eccentricity, but only 0.08 log units at 3.5 degrees eccentricity. Although this subject appeared to be an "over" responder, given that the mean increase in MPOD for their sample was only

about 0.10 log units at 30′ eccentricity, the observed changes in MPOD across the central retina may represent the pattern in which lutein and zeaxanthin are deposited in the macula. In fact, the same effect was observed in the current study. Mean increases at 20′ and 30′ eccentricity were both approximately 0.08 log units, whereas the mean increases at 60′ and 120′ eccentricity were only 0.04 and 0.01 log units, respectively. The mean increase of the Gaussian estimated peak after twelve weeks of intervention was 0.08; for the Lorentzian peak it was 0.09. Interestingly, for all four subjects in the current study, the coefficient of determination for both the Gaussian and Lorentzian fits of their MPOD profiles increased from baseline. Likewise, the post-intervention profile reported by Hammond et al. was better fit with a Gaussian (0.94 compared to 0.96) or Lorentzian (0.96 compared to 0.98) function than was the baseline profile. These findings suggest that MPOD does not increase uniformally across the fovea, but instead may follow a Gaussian or Lorentzian distribution.

One noteworthy difference between the current project and the study conducted by Hammond et al. (1997a) is the type of intervention: they used a food source of lutein, whereas a supplement was used in the current project. Although the changes in MPOD observed in the current study are similar to those of Hammond et al. (1997a) and other researchers that used natural sources of lutein (Johnson et al., 2000; Curran-Celentano et al., 2003), the supplement dosage of lutein required to achieve this effect was much greater. Few studies investigating the effects of lutein supplementation on MPOD found a significant effect using doses less than 20mg per day. For example, Lariviere et al. (2002) and Cardinault et al. (2003) failed to observe a significant change in MPOD after supplementation of 6mg and 9mg per day, respectively. One consideration, in addition to

the dose size, was the length of intervention in these studies. Both studies measured MPOD after 5 weeks of intervention. It may be that changes in MPOD occur after longer intervention periods when supplements, as opposed to natural sources, are used at doses below 10mg per day. Preliminary results from an intervention study in which subjects consumed 2.4mg of lutein per day suggest that a small dose may significantly increase MPOD in about sixty-percent of subjects over a six-month period (Bone et al., 2003). In contrast, Berendschot et al. (2000) claimed a significant increase in MPOD occurred after only four weeks of intervention with 10mg of lutein per day. Their results, however, should be viewed with caution, as the reported significance of the effect is clearly not supported by the data in their figures. The mean increase in MPOD for their small sample (n = 8) was approximately 0.01 log units after four weeks. Aleman et al. (2001) investigated the effects of a six-month intervention on MPOD in normal and diseased eyes. Their subjects consumed 20mg of lutein supplements daily for six months and had their MPOD measured at baseline and again after the intervention period. MPOD was measured using a Macular Metrics densitometer like the one used in the present study except their smallest target had a visual angle of 20′, as opposed to 40′. In normal subjects, MPOD significantly increased at 10′ eccentricity, but not at the three additional loci. Conversely, mean MPOD in patients with retinitis pigmentosa and Usher syndrome significantly increased at all four loci. In both groups, mean MPOD increased 0.07 at 10′ eccentricity, and in the diseased eyes, it increased 0.07, 0.08, and 0.04 at 30′, 60′ and 120′ eccentricity, respectively. Using the same paradigm, Duncan et al. (2002) reported similar changes in MPOD at these four loci. The significant changes in MPOD reported by Aleman et al. (2001) and Duncan et al. (2002) agree well with the significant changes

observed in the current study. If these researchers obtained more frequent measures of MPOD, a relationship between supplement dose and intervention length could possibly be estimated by comparing their data to the current project. It may be that the rate of increase of MPOD is identical in response to either 20mg or 30mg of lutein per day. This possibility is based on the findings of a few studies showing that increases in MPOD plateau after a certain point (e.g., Hammond et al., 1997a). Thus, the six-month profiles measured by Aleman et al. (2001) and Duncan et al. (2002) may represent MPOD that reached a saturation point after only twelve weeks of intervention. Alternatively, a longer intervention may be necessary to achieve a certain effect when using 20mg, compared to 30mg. For this possibility to be correct, the effects observed by Aleman et al. (2001) and Duncan et al. (2002) would have to occur after twelve-weeks of intervention, assuming the increases in these studies are similar to those in the current project.

Unfortunately, one cannot assess the interaction of dose and intervention length by comparing the data available to date (see Table 2). One study, however, investigated the effects of intervention length by obtaining frequent measures of MPOD during supplementation. Landrum et al. (1997) measured MPOD at 45′ eccentricity several times per week during supplementation with 30mg of lutein per day. Both subjects in this study responded to the treatment in a similar fashion. After a "lag" period of about three weeks, MPOD increased linearly throughout the 140 days of intervention. Their MPOD continued to increase for approximately thirty days after discontinuation of the supplement and remained at this elevated level for 200 days. The dosage used in the current project was selected based on the results of Landrum et al. (1997). In particular,

their subjects' MPOD increased approximately 0.15 log units after twelve weeks of intervention. This increase in MPOD is almost twice as high as the changes observed in the current study at two more central loci. If MPOD does increase more in central loci relative to peripheral loci (e.g., Hammond et al., 1997a), then one might expect that changes in MPOD at 20′ and 30′ eccentricity in the current project should have exceeded those of Landrum et al. (1997). The difference in effect size is difficult to reconcile given the similarities between the two studies (e.g., MPOD measured with HFP). However, one difference between the studies was the eccentricity of the reference measure of sensation luminance. In the current study, the extrafoveal measure was obtained at seven-degrees eccentricity, whereas Landrum et al. (1997) obtained a measure at eight-degrees eccentricity. Although it seems unlikely, if the mean MPOD of subjects in the current study increased 0.07 at six degrees eccentricity, then their veridical increase in MPOD would be about the same as Landrum et al.'s (1997) subjects. A more plausible explanation is that the four individuals in the current project may be "average" responders, whereas Landrum et al.'s (1997) two subjects may be "over" responders, like the one described by Hammond et al. (1997a).

Taken together, the results of the various intervention studies suggest that lutein intervention, with either natural sources or supplements, can increase MPOD *approximately* 0.10 log units at loci inside one-degree eccentricity. While MPOD appears to increase more in some subjects relative to other responders, a few subjects do not appear to respond to intervention. In addition to the aforementioned dietary factors that affect lutein bioavailability, a few factors may limit the effects of lutein intervention on MPOD. As previously mentioned, the duration of lutein intervention may affect

changes in MPOD. If MPOD does not increase during the first three to four weeks of intervention, as reported by Landrum et al. (1997), it is not surprising that studies with short intervention periods failed to observe a significant change in MPOD (e.g., Lariviere et al., 2002; Cardinault et al., 2003). Another possible limiting factor may be a subject's baseline MPOD. Assuming the retina has a limited number of binding sites for carotenoids, it seems logical that individuals with high MPOD have fewer free binding sites than individuals with low MPOD. Thus, one might expect that an individual with low MPOD might respond more to intervention than an individual with high MPOD. In the current project, however, the increases in MPOD were the same for subject AW, who had a baseline MPOD of 0.51 at 30′ eccentricity, and subject LS, who had a baseline MPOD of 0.15 at this eccentricity. The independence of baseline MPOD and subsequent increases in MPOD was also noted by Bone et al. (2003). A related limiting factor may be the ceiling effects observed in some studies. Hammond et al. (1997a), for example, found that MPOD appeared to plateau after four weeks of intervention, despite an additional month of dietary modification. It may be that retinal lutein and zeaxanthin have a saturation point, such that concentrations beyond this threshold fail to yield measurable increases in MPOD. In an in vitro model, Junghans et al. (2001) showed that as the lutein or zeaxanthin concentration in liposomes increased, light transmission through the medium decreased exponentially and eventually asymptotes. One might assume that such a saturation effect might not only affect measures of MPOD in an intervention study, but also PP.

Macular Pigment Optical Density and Photophobia

The results of the current project suggest that MPOD plays a role in PP thresholds. In Experiment One, there was a significant linear relationship between PP ratio and MPOD at 20′, 30′ and 60′ eccentricity. Individuals with higher MPOD at these loci required more SW light, relative to MW light, to experience PP in the fovea. In other words, the amount of SW light filtered by subjects' MP was directly proportional to their PP ratios. The relationship between SW light absorption at 20′ eccentricity and PP ratio was such that a ten-percent change in light transmission resulted in a 0.07 change in PP ratio. The correlation between PP ratio and MPOD was strongest for the 20′ locus. Typically, individuals with high MPOD at central loci tend to have higher aggregate totals of MP. This relationship was true for nine of the ten subjects in Experiment One. The one subject who did not fit the correlation had relatively high MPOD at 60′ and 120′ eccentricity, compared to his MPOD at 20′. Such individuals demonstrate that measures of MPOD at a single locus cannot accurately estimate aggregate MP (Aleman et al., 2001; Robson et al., 2003). In fact, this subject's MPOD at 20′ eccentricity was the third lowest in the sample, but his integrated MPOD was approximately the same as the subject with the highest MPOD at 20′ eccentricity. Given the similarity in total MP screening for these two subjects, it is not surprising that they had similar PP ratios. A similar instance was noted by Stringham et al. (2004). In their study, two individuals had distinctly different MPOD profiles, but almost identical iLMPOD and PP thresholds. This finding supports the idea that PP thresholds are affected by the aggregate screening of MP across the fovea (Stringham et al., 2003; 2004). Indeed, the correlations between PP ratio and iGMPOD and iLMPOD are stronger than the relationships between PP ratio

and MPOD at any single locus. This difference in the strength of the correlation would undoubtedly be larger if the sample contained more individuals with broad or mesokurtic MPOD profiles. Still, the significant correlations between PP ratio and MPOD suggest that these two phenomena are related.

In Experiment Two, repeated measures of MPOD and PP ratio were obtained during an intervention with lutein supplements. After twelve-weeks of supplementation, MPOD at the three central loci, as well as iGMPOD and iLMPOD, significantly increased from baseline. The significant mean increase in SW light absorption resulted in a significant increase in mean PP ratio. This finding suggests that MP can affect PP thresholds for lights composed of wavelengths within its absorption spectrum. The relationship between increases in integrated MPOD and PP ratio was such that each ten-percent increase in integrated MPOD corresponded to a 0.05 increase in PP ratio. In terms of supplementation, a 0.05 increase in PP ratio was observed after subjects consumed a total of 1,260mg of lutein. When the data from Experiment Two are added to the bivariate correlations in Experiment One, the strength of the relationship between PP ratio and iGMPOD ($p < 0.0001$) and iLMPOD ($p < 0.0001$) increases, although the additional eight data points are not independent measures. The function observed in Experiment One predicated the changes in PP ratio observed in Experiment Two. Thus, Experiment Two not only upheld the correlation measured in Experiment One, but demonstrated that MP may affect PP thresholds for SW lights.

The results of the current project may be relevant to some clinical conditions. As stated in the Introduction, PP is a common symptom of migraine headaches. The prevalence of recurrent migraine headaches may be as high as eighteen-percent of the

population, and studies report that between 50 and 100 percent of patients experience PP (Vanagaite et al., 1997; Krymchantowski and Moreira 2001; Wöber-Bingöl et al., 2004). Lutein supplementation, or increased MP, may not prevent episodes of PP, but it may increase patients' PP thresholds, allowing them to function (with visual comfort) under a broader range of light intensities. Certain eye diseases, such as AMD and cataract, are also associated with an increased incidence of PP. Lutein supplementation, then, may be beneficial for some patients to not only preserve ocular health, but increase PP thresholds. Relatedly, Olmedilla et al. (2001) showed that lutein supplementation (9mg per week) significantly improved glare thresholds in patients with cataract. The patient's increased consumption of lutein likely increased their MP, attenuating the amount of SW light contributing to their glare thresholds post-treatment, relative to baseline. The post-treatment glare thresholds were measured after an average of twenty months of intervention. One noteworthy finding in the current project was that PP thresholds could be significantly increased in less than three months. The collective results of Olmedilla et al. (2001) and the current project suggest that lutein supplementation may augment MP, and thereby increase thresholds of visual discomfort in patients.

Stringham et al. (2003) proposed that PP acts as an inherent mechanism to protect the retina from potentially dangerous levels of light. This postulation was predicated on their finding that PP thresholds were inversely related to wavelength, as is energy, and appeared to parallel the retinal damage function measured by Ham et al. (1976). Their inference seems reasonable, as one would expect a photo-protective mechanism to be biased towards more damaging wavelengths of light. Additionally, one might expect that PP magnitude, or degree of visual discomfort, might be correlated with the intensity of

the light source. This assumption is based on the idea that some lights, while discomforting, may pose less risk for damage than more intense lights. In the former instance, an adequate photo-protective response may be a moderate squint, whereas the latter instance might require a large squint or eye closure. In the studies performed by Stringham et al. (2003, 2004), some lights exceeded the 4:1 signal-to-noise ratio criterion for PP. Similarly, Deaver et al. (1996) showed that the length of the palpebral fissure was inversely related to luminance. These findings are further supported by measures of visual discomfort using scaling techniques (Sivak et al., 1999). If the magnitude or threshold of visual discomfort is affected by light intensity, as these studies suggest, it follows that an intervening filter should influence visual discomfort thresholds. In outdoor environments, for example, sunglasses may reduce visual discomfort or squint magnitude (Sliney, 2002). Likewise, the current project demonstrates that a SW filter, the MP, attenuates PP for SW lights. The results of Stringham et al. (2004) suggest that MP also affects thresholds for broad-band targets containing SW light (Stringham et al., 2004). Given the influence of MP on PP, it may be that these two phenomena operate jointly to protect the retina from excessive levels of light. The MP may act as a static mechanism, constantly screening light in the more harmful range of the visible spectrum, whereas PP may act as a dynamic mechanism, occurring when retinal illuminance reaches or exceeds a certain risk threshold. In addition to protecting the retina from potential light-induced damage, MP and PP may act to mitigate transitory deficits of vision caused by afterimages. One anecdotal observation made by Stringham and colleagues in their research on PP (but not yet reported) was that SW lights tended to produce much longer afterimages than MW and LW lights containing less energy.

CHAPTER IX

FUTURE DIRECTIONS

The results of the current project connected two phenomena that scientists have been investigating separately for more than a century. Still, numerous factors associated with MP and PP are unrealized. If MP improves visual function in diseased eyes or can inhibit the progression of retinal degeneration, it is important to determine the dose of carotenoids and intervention duration necessary to affect visual performance or reduce one's risk for AMD. This issue is particularly important in light of the growing number of commercially available lutein supplements and the lack of a Food and Drug Administration Recommended Daily Allowance for lutein and zeaxanthin. If the retinal carotenoids are conditionally essential for the health of the macula (Semba & Dagnelie, 2003), it is also important to determine why some individuals have extremely low MPOD, and relatedly, why some individuals show no retinal response to lutein intervention. These individuals may be at greater risk for developing macular disease.

The symptomatology of AMD, as well as several other age-related eye diseases, includes PP. Yet, most research investigating the clinical aspects of PP has focused on its prevalence in patients with migraines or neurological disorders. Using the methodology in the current project, it would be valuable to investigate the effects of lutein intervention on PP thresholds in a clinical sample. If the results of such a study are similar to the current project's findings, lutein supplementation may be beneficial for individuals with frequent episodes of PP.

Research investigating the stimulus conditions that induce PP might also be useful. If SW light is more likely to induce PP than MW or LW light, then an individual with frequent episodes of PP might benefit from using household lighting devoid of SW light. Unfortunately, previous researchers, except for Stringham et al. (2003; 2004), failed to control or quantify retinal illuminance in their studies of the stimulus conditions that affect PP thresholds. Thus, it may be beneficial to retest some of their hypotheses using Maxwellian view optics. Additionally, several other conditions that might influence PP thresholds, such as the eye's state of adaptation, are unexplored.

CHAPTER X

CONCLUSION

Numerous studies suggest that retinal lutein and zeaxanthin, or MP, may play a role in retinal health and visual performance. The antioxidant properties of the retinal carotenoids may attenuate oxidative stress, while the absorbency of MP may mitigate light stress and concurrently reduce chromatic aberration. The light filtering properties of MP also appear to influence visual comfort. Specifically, the current project demonstrated that the MP increases the amount of light necessary to induce visual discomfort, or PP for SW targets. Should this prove beneficial, it has been demonstrated that twelve weeks of lutein supplementation can significantly increase MPOD, and as a result, PP thresholds.

CHAPTER XI

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CHAPTER XIII

APPENDIX A

Table 1. Age, Sex, BMI and MPOD of Experiment One subjects.

				MPOD			
Subject	Sex	Age	BMI	20′	30′	60′	120′
JPS	male	24	25.5	0.476	0.400	0.204	0.013
LS	female	27	19.6	0.209	0.152	0.101	0.032
JMS	male	32	24.4	0.354	0.258	0.179	0.016
AW	male	30	22.6	0.656	0.513	0.195	0.056
MW	female	29	21.3	0.415	0.314	0.213	0.126
MV	female	21	22.2	0.569	0.397	0.199	0.065
ML	male	29	29.6	0.410	0.376	0.260	0.101
CF	female	26	20.0	0.143	0.059	0.061	0.077
SB	female	21	26.6	0.513	0.394	0.230	0.019
BJ	female	26	24.1	0.483	0.401	0.216	0.145
	mean	26.5	23.6	0.423	0.326	0.186	0.065
	SD	3.68	3.10	0.156	0.135	0.060	0.047
	CV	13.8	13.1	36.8	41.4	32.2	72.3

CHAPTER XIII

APPENDIX B

Table 2. Lutein Intervention Studies that Measured MPOD in normal subjects.

Study[1]	# of Subjects	Lutein Dose Per Day	Intervention (weeks)	Retinal Loci Measured	Mean Change in MPOD[2]	p
A[3]	10	11.2mg	15	30′	0.11	< 0.05
B	2	30mg	20	45′	0.23	< 0.005
C[4]	8	10mg	12	1.5° field	0.01	< 0.001
D[3]	7	11.2mg	15	30′	0.07	< 0.05
E	8	20mg	26	20′	0.07	0.04
				30′	0.01	0.53
				60′	0.03	0.11
				120′	0	0.79
F	4	20mg	26	20′	0.09	0.002
				30′	nr	0.56
				60′	nr	0.35
				120′	nr	0.66
G[5]	7	6mg	4	20′	nr	nr
				30′	nr	nr
				60′	nr	nr
				120′	nr	nr
H[6]	21	2.4mg	26	45′	0.04	< 0.05
I[4, 5]	29	9mg	5	2° field	nr	nr
J[3]	6	214μg[7]	12	30′	0.11	< 0.04

[1] Study: A, Hammond et al (1997a); B, Landrum et al (1997); C, Berendschot et al (2000); D, Johnson et al (2000); E, Aleman et al (2001); F, Duncan et al (2002); G, Lariviere et al (2002); H, Bone et al (2003); I, Cardinault et al (2003); J, Curran-Celentano et al (2003)

[2] nr = not reported.

[3] Used a natural source of lutein (A, D: spinach + corn; J: eggs), all other studies used lutein supplements.

[4] Measured MPOD with reflectometry, yielding an average MPOD across a foveal area.

[5] No significant change in MPOD at any eccentricity was observed.

[6] Significant increases in MPOD were only observed for 12 of the 21 subjects.

[7] Calculated daily average of consuming six eggs per week.

CHAPTER XIV

APPENDIX C

University of New Hampshire Institutional Review Board approval letter.

UNIVERSITY of NEW HAMPSHIRE

July 23, 2004

Wenzel, Adam
Psychology, Conant Hall
25A E. Concord, Apt. #3
Dover, NH 03820

IRB #: 2990
Study: Impact of Macular Pigment on Photophobia
Approval Date: 07/24/2003

The Institutional Review Board for the Protection of Human Subjects in Research (IRB) has reviewed and approved the protocol for your study as Expedited as described in Title 45, Code of Federal Regulations (CFR), Part 46, Subsection 110.

Approval is granted to conduct your study as described in your protocol for one year from the approval date above. At the end of the approval period, you will be asked to submit a report with regard to the involvement of human subjects in this study. If your study is still active, you may request an extension of IRB approval.

Researchers who conduct studies involving human subjects have responsibilities as outlined in the attached document, *Responsibilities of Directors of Research Studies Involving Human Subjects.* (This document is also available at http://www.unh.edu/osr/compliance/IRB.html.) Please read this document carefully before commencing your work involving human subjects.

If you have questions or concerns about your study or this approval, please feel free to contact me at 603-862-2003 or Julie.simpson@unh.edu. Please refer to the IRB # above in all correspondence related to this study. The IRB wishes you success with your research.

For the IRB,

Julie F. Simpson

Julie F. Simpson
Manager

cc: File
Kenneth Fuld

Research Conduct and Compliance Services, Office of Sponsored Research, Service Building, 51 College Road, Durham, NH 03824-3585 * Fax: 603-862-3564